CENTRAL LUZON STATE UNIVERSITY

# SINGLE CELL PROTEIN POTENTIAL OF ENDOPHYTIC FUNGI ASSOCIATED WITH BAMBOO USING DRIED ONION STALK AS SUBSTRATE

**LEVI P. CORPUZ**

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 Fulfilment of the Requirements for the Degree

**BACHELOR OF SCIENCE IN BIOLOGY**

**JUNE 2017**

Republic of the Philippines
**CENTRAL LUZON STATE UNIVERSITY**
Science City of Muñoz, Nueva Ecija

COLLEGE OF ARTS AND SCIENCES
Department of Biological Sciences

**APPROVAL SHEET**

The undergraduate thesis entitled; **SINGLE CELL PROTEIN POTENTIAL OF ENDOPHYTIC FUNGI ASSOCIATED WITH BAMBOO USING DRIED ONION STALK AS SUBSTRATE** prepared and submitted by **LEVI P. CORPUZ** in partial fulfilment of the requirements for the degree **BACHELOR OF SCIENCE IN BIOLOGY** is hereby approved and accepted.

**MARY JHANE G. VALENTINO, M. Sc.**
Adviser

Date Signed

**EDEN S. DAVID, Ph.D.**
Critic

Date Signed

**RICH MILTON R. DULAY, M.Sc.**
Department Research Coordinator

Date Signed

Accepted in partial fulfilment of the requirements for the degree of **BACHELOR OF SCIENCE IN BIOLOGY.**

**EVARISTO A. ABELLA, Ph.D.**
Department Chair

Date Signed

**ANNA MARIA LOURDES S. LATONIO, Ph.D.**
College Research Coordinator

Date Signed

**MYRNA R. UMAGAT, Ph.D.**
Dean

Date Signed

## BIOGRAPHICAL SKETCH

### PERSONAL INFORMATION

| | |
|---|---|
| Name: | LEVI P. CORPUZ |
| Date of birth: | March 09, 1998 |
| Place of birth: | Bongabon, Nueva Ecija |
| Address: | Brgy. Social Bongabon Nueva Ecija |
| Religion: | Roman Catholic |
| Parents: | Mr. Eriberto P. Corpuz |
| | Mrs. Minifreda P. Corpuz |

### EDUCATIONAL BACKGROUND

| | | |
|---|---|---|
| *TERTIARY* (A.Y. 2013-2017) | : | Central Luzon State University<br>Science City of Muñoz, Nueva Ecija<br>Bachelor of Science in Biology |
| *SECONDARY* (A.Y. 2009-2013) | : | Nueva Ecija High School<br>Burgos Street Cabanatuan City |
| *ELEMENTARY* (A.Y. 2002-2009) | : | Rosa A. Ilagan Elementary School<br>Brgy. San Roque Bongabon Nueva Ecija |

### SEMINARS ATTENDED

Symposium on "Direct Seeding; A Response to Economical and Technological Challenges in Rice Farming" CLSU Auditorium, Science City of Munoz, Nueva Ecija, Philippines (Septembet 7, 2013)

Symposium on "Current Trends in Food Safety and Quality assurance" CLSU Auditorium, Science City of Munoz, Nueva Ecija Philippines (August 09, 2017)

Symposium on HIV: Aids "Survival of the fittest, Human living culture medium CLSU Auditorium, Science City of Munoz, Nueva Ecija

Symposium on"Philippine Biodiversity and the National Museum." CLSU, Auditorium, Science of City Munoz, Nueva Ecija, Philippines (March 22, 2016)

**ON THE JOB TRAINING**

Bureau Of Fisheries And Aquatic Resources (REGION 1) @Brgy. Lucap Alaminos, Pangasinan Philippines June-July 2016

## ACKNOWLEDGEMENT

The author would like to express his sincere and deepest gratitude to his Thesis Adviser, Ms. Mary Jhane G. Valentino for her patience, motivation, willingness, and for not giving up on his advisee. Her non-stop perseverance and advices were unforgettable. If the author were to choose another thesis adviser again, he would still choose Ms. Jhane G. Valentino.

To the author's critic Mrs. Eden S. David, thank you for the suggestions, recommendations and other help to improve the study. To the Department Research Coordinator, Mr. Rich Milton R. Dulay, a sincere thanks is given for the help that he provided to the author. You will always be remembered.

The author would also like to thank all of his BS BIO FRIENDS and DORMMATES, for their support, thoughtfulness and understanding along the way. Deep appreciation and thoughtful gratitude for the help of his acquaintances, KUYA DENNIS, BFAR LUCAP, and JAEC SANTIAGO for the unmeasurable help they provided.

The author would also like to thanks his Relatives in Bongabon, Nueva Ecija specially his grandmother Mrs. Nora P. Marin for her unconditional support, guidance, love, and for always reminding him to not give up on his dreams and goals in life. The author would also like to expound his very special heartfelt thanks to his PARENTS, Mr. Eriberto P. Corpuz and Mrs. Minifreda P, Corpuz who never gave up on their son. Their love, patience, faith, will always be appreciated and valued.

Most of all, the author would like to thank Lord God in his unconditional love, blessings and un-measurable love to the author in order to be safe and to live life every other day. Thank you Lord for everything you have done for me and for everything you will yet to give in my life! Thank you very much! Glory to GOD!

LEVI PADILLA CORPUZ

# TABLE OF CONTENTS


| | PAGE |
|---|---|
| TITLE PAGE | i |
| APPROVAL SHEET | ii |
| BIOGRAPHICAL SKETCH | iii |
| ACKNOWLEDGEMENT | v |
| TABLE OF CONTENTS | vii |
| LIST OF TABLES | ix |
| LIST OF APPENDICES | x |
| LIST OF APPENDIX TABLE | xi |
| LIST OF APPENDIX FIGURE | xii |
| ABSTRACT | xiii |
| INTRODUCTION | |
| Background of the Study | 1 |
| Objectives of the Study | 2 |
| Significance of the Study | 3 |
| Scope and Limitations of the Study | 3 |
| Time and Place of the Study | 4 |
| REVIEW OF RELATED LITERATURES | 5 |
| Single Cell Protein | 5 |
| Production of SCP | 7 |
| Microorganisms and Substrates used for SCP Production | 9 |
| Substrates for SCP Production | 11 |
| Crude Protein Content | 12 |
| Fungal Biomass | 12 |

| | |
|---|---|
| Biomass Production | 13 |
| Livestock Feed | 14 |
| Endophytic Fungi | 16 |
| Aflatoxin | 19 |
| *Aspergillus* | 19 |
| Onion Wastes | 21 |
| Solid State Fermentation | 21 |
| **MATERIALS AND METHODS** | 24 |
| Preparation of the Inocula | 25 |
| Preparation of Substrates | 25 |
| Inoculation of Fungal endophytes in Onion Stalk | 25 |
| Harvesting and Drying | 26 |
| Proximate Composition | 26 |
| Data Gathered | 27 |
| Statistical Analysis | 27 |
| **RESULTS AND DISCUSSION** | 28 |
| Crude Protein Content of Fungal Endophytes treated onion stalk | 28 |
| Proximate Composition of Fungal Enriched Dried Onion Stalk | 30 |
| Moisture Content | 31 |
| Ash content | 32 |
| Crude fiber content | 33 |
| Crude fat content | 34 |
| **SUMMARY, CONCLUSION AND RECOMMENDATION** | 36 |
| Summary | 36 |
| Conclusion | 37 |
| Recommendation | 38 |
| **LITERATURE CITED** | 39 |
| **APPENDICES** | 44 |

# LIST OF TABLES


| TABLE | | PAGE |
|---|---|---|
| 1 | Treatments for Single Cell Protein | 24 |
| 2 | Percent increase in crude protein content | 30 |
| 3 | Mean percentage of proximate composition of enriched onion stalk | 32 |

# LIST OF APPENDICES


| APPENDIX | | PAGE |
|---|---|---|
| A | Statistical Result | 45 |
| B | Proximate Analysis Result | 47 |

# LIST OF APPENDIX TABLES


| APPENDIX TABLE | | PAGE |
|---|---|---|
| 1 | One Way ANOVA of crude protein percentage | 45 |
| 2 | One Way ANOVA of increase in crude protein percentage | 45 |
| 3 | One Way ANOVA of moisture percentage | 45 |
| 4 | One Way ANOVA of ash percentage | 46 |
| 5 | One Way ANOVA of crude fiber percentage | 46 |
| 6 | One Way ANOVA of crude fat percentage | 46 |




LEVI P. CORPUZ
Bs Biology 2017

## LIST OF APPENDIX FIGURES


| APPENDIX FIGURE | | PAGE |
|---|---|---|
| 1 | Proximate Analysis Result (Control) | 47 |
| 2 | Proximate Analysis Result (*Aspergillus niger*) | 48 |
| 3 | Proximate Analysis Result (*Aspergillus flavus*) | 59 |
| 4 | Proximate Analysis Result (*Aspergillus ochraceus*) | 50 |
| 5 | Proximate Analysis Result (*Cladosporium cladosporioides*) | 51 |
| 6 | Proximate Analysis Result (*Fusarium sp.1*) | 52 |
| 7 | Proximate Analysis Result (*Fusarium sp.2*) | 53 |
| 8 | Proximate Analysis Result (*Fusarium semitectum* | 54 |
| 9 | Proximate Analysis Result (*Monascus ruber*) | 55 |
| 10 | Proximate Analysis Result (*Penicillium citrinum*) | 56 |

## ABSTRACT

**CORPUZ LEVI P.,** Bachelor of Science in Biology, Department of Biological Sciences, College of Arts and Sciences, Central Luzon State University, Science City of Munoz, Nueva Ecija, Philippines, June 2017, **SINGLE CELL PROTEIN POTENTIAL OF ENDOPHYTIC FUNGI ASSOCIATED WITH BAMBOO USING DRIED ONION STALK AS SUBSTRATE**

**Manuscript No: BIO-M-2nd17-024**

**Adviser: Mary Jhane G. Valentino, M.Sc.**

This study was conducted to determine the ability of nine endophytic fungi associated with bamboo namely; *Aspergillus niger, Aspergillus flavus, Aspergillus ochraceous, Fusarium* sp.1, *Fusarium* sp.2, *Fusarium semitectum, Monascus ruber, Penicillium citrinum,* and *Cladosporium cladosporoides* in the production of single cell protein using onion stalk as substrate. Single cell protein potential of the nine fungal endophytes were evaluated through the increase in crude protein content of the fungal enriched onion stalk. Additionally, proximate composition of the fungal enriched dried onion stalks was also tested.

Results revealed the capability of the nine endophytic fungi in single cell protein production using dried onion stalks as substrates which led to the increased CPC of the dried onion stalk. *P. citrinum* - treated dried onion stalk exhibited the highest CPC of 19.06% while *A. niger* - treated dried onion stalk had the least of 16.87%. As for the proximate composition analysis, reduction of the moisture content in all fungi treated dried onion stalks was assessed after 20 days of solid state fermentation. Increase in ash

content and crude fiber of all fungi treated dried onion stalks were also noted. Meanwhile, reduction in the crude fat content of dried onion stalks treated with A. *flavus, M. ruber, F. semitectum, A. ochraceous, Fusarium sp.2, A. niger* and *Fusarium sp.1* were observed while the rest of the fungal endophytes increased the crude fat of the substrate.

## LITERATURES CITED

**ADEDAYO, M.R., AJIBOYE, E.A., AKINTUNDE,. KODAIBO, J.K., and A. PRL. (2011).** Single Cell Proteins: As Nutritional Enhancer. Advances in Applied Science Research2 (5):396-409.

**ADEYEMI, O.T., MUHAMMAD, N.O., OLADIJI, and A.T. (2012).** Biochemical assessment of the mineral and some anti-nutritional constituents *of Aspergillus niger* fermented *Chrysophyllum albidum* seed meal. African Journal of Food Science, 6(1): 20-28.

**ALEXANDRATOS, N., and J. BRUINSMA. (2012).** World agriculture towards 2030/2050: the Rome, Food and Agricultural Organization: 2012 revision (No. 12-03, p. 4).

**AL-MHANNA, N. (2011).** Single Cell Protein (SCP) Production from Date Juice (Doctoral dissertation, Universitätsbibliothek der Universität Erlangen-Nürnberg).

**BEIGH, Y.A., GANAI, A. M., and H.A. AHMAD. (2015).** Utilization of Apple pomace as livetock feed: A review. Indian Journal of Small Ruminants (The), 21(2): 165- 179.

**BENÍTEZ, V.M., MARTÍN, E.C., AGUILERA, M.A., LÓPEZ, A.Y., COOLS, F.J., and R.M. ESTEBAN. (2011).** Characterization of industrial onion wastes (*Allium cepa L.*): dietary fibre and bioactive compounds. Plant foods for human nutrition, 66(1): 48-57.

**BHALLA, T.C., SHARMA, N.N., and M. Sharma. (2007)**. Production of metabolites, industrial enzymes, amino acid, organic acids, antibiotics, vitamins and single cell proteins. Department of Biotechnology. Himachal Pradesh University.

**CHANDRANI-WIJEYARATNE, S., and A.N. TAYATHILAKE. (2000).** Characteristics of two yeast strain (*Candida tropicalis*) isolated from *Caryota urens* (Khitul) toddy for single cell protein production. Journal National Science Foundation Sri Lanka, 28: 79-86.

**CLAY, K. and C. SCHARDL. (2002).** Evolutionary origins and ecological consequences of endophyte symbiosis with grasses. The American Naturalist, 160(S4): S99-S127.

**DOS SANTOS, O.M., FEDDERN, V.K., CIPOLATTI, E.P., BADIALE, F.E., and L.A. DE SOUZA-SOARES. (2011).** Changes in lipid, fatty acids and phospholipids composition of whole rice bran after solid- state fungal fermentation. Bioresource technology, 102(17): 8335-8338.

**FAETH, S.H. (2002).** Are endophytic fungi defensive plant mutualists?. Oikos, 98(1): 25-36.

**GLAZER, A.N. (1994).** Phycobiliproteins—a family of valuable, widely used fluorophores. Journal of Applied Phycology, 6(2): 105-112.

**HAIDER, M.M., EL-TAJORIS, N.N., and S.H. BAIU. (2003).** Single cell protein production from carob pod extracts by the yeast Saccharomyces cerevisiae. Faculties of Science and Medical Research 5(3): 209-214.

**HESSELTINE, C.W., SHOTWELL, O.L., ELLIS, J.J., and R.D. STUBBLEFIELD. (1966).** Aflatoxin formation by Aspergillus flavus. Bacteriological reviews, 30(4): 795.

**HÖLKER, U., HÖFER, M., and J. LENZ. (2004).** Biotechnological advantages of laboratory-scale solid-state fermentation with fungi. Applied Microbiology and Biotechnology, 64(2): 175-186.

**HSIEH, C., and F.C. YANG. (2004).** Reusing soy residue for the solid-state fermentation of Ganoderma lucidum. Bioresource Technology, 91(1): 105-109.

**JOSHI, V.K., and D.K. SANDHU. (1996).** Preparation and evaluation of an animal feed byproduct produced by solid-state fermentation of apple pomace. Bioresource Technology, 56(2-3): 251-255.

**KANNADAN, S., and J.A. RUDGERS, (2008).** Endophyte symbiosis benefits a rare grass under low water availability. Functional Ecology, 22(4): 706-713.

**KHAN, M.K., AHMED, S.A.,, and A.Z. TANVEER. (2010).** Production of single cell protein from *Saccharomyces cerevisiae* by utilizing fruit wastes. Nanobiotechnica Universale, 1(2): 127-132.

**KHAN, S.A., HAMAYUN, M.Y., KIM, H., SUH, H.Y.,, HWANG, S.J., and W.S. KONG. (2008).** Plant growth promotion and Penicillium citrinum. Bio Med Central Microbiology, 8(1): 231.

**KOBAYASHI, D.Y., and J.D., PALUMBO, (2000).** Bacterial endophytes and their effects on plants and uses in agriculture. Microbial endophytes, 19: 199-233.

**KOSSEVA, M.R. (2013).** Food Industry Wastes: Chapter 5. Recovery of Commodities Food Wastes Using Solid-State Fermentation. Elsevier Incorporated Chapters. ISBN: 978-0-12-391921-2.

**KRISHNA, C. (2005).** Solid-state fermentation systems—an overview. Critical reviews in biotechnology, 25(1-2): 1-30.

**LARDY, G., and V. ANDERSON. (2009).** Alternative feeds for ruminants. North Dakota State University AS: 1182.

**LICHTFIELD, J.H.(1980).** Microbial Protein Production. Bioscience 30, n0.6 (1980):387-396

**MORAKOTKARN, D., KAWASAKI, H., and T. SEKI. (2007)**. Molecular diversity of bamboo-associated fungi isolated from Japan. Federation of European Microbiological Societies microbiology letters, 266(1): 10-19.

**NASSERI, A.T., RASOUL, A.S., MOROWVAT, M.H., and Y. GHASEMI. (2011).** Single cell protein: production and process. American Journal of food technology, 6(2): 103-116.

**OBOH, G., and A.A. AKINDAHUNSI, (2005).** Nutritional and toxicological evaluation of Saccharomyces cerevisae fermented cassava flour. Journal of Food Composition and Analysis, 18(7): 731-738.

**OJEBIYI, O.O., OLADITI, O.A., OLADUNJOYE, I.O., and T.A. RAFIU. (2014).** Performance, Nutrient Utilization and Carcass Characteristics of Harco Cockerels Fed Diets Containing Bio-Treated Cassava Peels Meal at Chick Phase. Journal of Animal Production Research, 26(1).

**PANDEY, A., SOCCOL, C.R., and D. MITCHELL. (2000).** New developments in solid state fermentation: I-bioprocesses and products. Process biochemistry, 35(10): 1153-1169.

**PAYNOR, K.A., DAVID, E.S., and VALENTINO, M.J.G. (2016).** Endophytic fungi associated with bamboo as possible sources of single cell protein using corn cob as a substrate. Mycosphere, 7(2): 139-147

**PIMENTEL, M.R., MOLINA, G.D., MARÓSTICA A.P., and M.R. PASTORE. (2010).** The use of endophytes to obtain bioactive compounds and their

application in biotransformation process. Biotechnology research international, vol. 2011, Article ID 576286, 11 pages, 2011. doi:10.4061/2011/576286

**SANUSI, G.O., BELEWU, M.A., and B.O. ODUGUWA. (2013).** Dietary Effects of Solid- State Fermented *Jatropha curcas* Kernel Cake on West African Dwart Goats in a Mixed Ration. Environmental Resources and Agricultural Research, 14(2): 448-455.

**SCHUSTER, E., DUNN-COLEMAN, N., FRISVAD, J.C., and P. VAN DIJCK, (2002).** On the safety of *Aspergillus niger*–a review. Applied microbiology and biotechnology, 59(4-5): 426-435.

**SOCCOL, C.R., DA COSTA, E.S.F., LETTI, L.A.J., KARP, S.G., WOICIECHOWSKI, A.L., and L.P. DE SOUZA VANDENBERGHE. (2017).** Recent developments and innovations in solid state fermentation. Biotechnology Research and Innovation. Bioprocess Engineering and Biotechnology Department, Federal University of Paraná, 81531-970.

**SLIPPERS, B., and M.J. WINGFIELD. (2007).** Botryosphaeriaceae as endophytes and latent pathogens of woody plants: diversity, ecology and impact. Fungal biology reviews, 21(2): 90-106.

**SMIL, V. (2002).** Nitrogen and food production: proteins for human diets. AMBIO: *A* Journal of the Human Environment, 31(2): 126-131.

**STONE, J.K., BACON, C.W., and J.F. WHITE. (2000)**. An overview of endophytic microbes: endophytism defined. Microbial endophytes, 3: 29-33.

**SUMAN, G.N., ANURADHA, S.M., and B. PRADEEP. (2015).** Single cell protein production: a review. International Journal for Current Microbiology Application Science 4(9), 251-262

**SWEENEY, M.J., and A.D. DOBSON. (1999).** Molecular biology of mycotoxin biosynthesis. FEMS Microbiology Letters, 175(2), 149-163.

**TAN, R.X., and W.X. ZOU. (2001).** Endophytes: a rich source of functional metabolites. Natural product reports, 18(4), 448-459.

**TOPE, A.K. (2014).** Effect of fermentation on nutrient composition and anti-nutrient contents of ground Lima bean seeds fermented with *Aspergillus fumigatus*, *Rhizopus stolonifer* and *Saccharomyces cerevisiae*. International Journal of Advanced Research, 2(7): 1208-1215.

**UGALDE, U., J. CASTRILLO. (2002).** Single Cell Proteins from Fungi and Yeasts. Applied Mycology and Biotechnology, 2, 123-149.

**VALENTINO, M.J.G., SANTIAGO, J.C., and M. SALVADOR. (2016).** Protein enrichment of rice straw and sugarcane bagasse with endophytic fungi associated with bamboo using solid state fermentation. International Journal of Biology, Pharmacy and Allied Sciences, 5(6): 1270-1279.

**VANDENBERGHE, L. P., SOCCOL, C. R., PANDEY, A., and J.M. LEBEAULT. (2000).** Solid-state fermentation for the synthesis of citric acid by Aspergillus niger. Bioresource Technology, 74(2): 175-178.

**WADHWA, M., and M.P.S. BAKSHI, (2013).** Utilization of fruit and vegetable wastes as livestock feed and as substrates for generation of other value-added products. Food and Agriculture Organizational, ISBN 978-92-5-107631-6.

**YANG, S.S. (1988).** Protein enrichment of sweet potato residue with amylolytic yeasts by solid-state fermentation. Biotechnology and Bioengineering, 32(7): 886-890.