ISOLATION AND CHARACTERIZATION OF MUNGBEAN RHIZOBIA GROWN FROM SELECTED SOILS IN WESTERN PANGASINAN

ALFRED KONRAD G. AQUINO

An undergraduate thesis manuscript submitted to the faculty of Department of Soil Science College of Agriculture, Central Luzon State University in partial fulfillment of the requirements for the degree of

BACHELOR OF SCIENCE IN AGRICULTURE
(Soil Science)

June 2017

ISOLATION AND CHARACTERIZATION OF MUNGBEAN RHIZOBIA GROWN FROM SELECTED SOILS IN WESTERN PANGASINAN

by

ALFRED KONRAD GARIN AQUINO

An undergraduate thesis manuscript presented to the faculty of Department of Soil Science, College of Agriculture, Central Luzon State University, Science City of Muñoz in partial fulfillment of the requirements for the degree of Bachelor of Science in Agriculture major in Soil Science

APPROVED:

FLORIDA C. GARCIA
Adviser
6/10/17
Date Signed

PURISIMA P. JUICO
Critic
6/14/17
Date Signed

FEDERICO O. PEREZ
Department Research Coordinator
06-13-17
Date Signed

PURISIMA P. JUICO
Department Chairperson
6/14/17
Date Signed

ACCEPTED:

ERNESTO A. MARTIN
Dean
6-14-17
Date Signed

RECORDED:

RONALDO T. ALBERTO
College Research Coordinator
6/14/17
Date Signed

BIOGRAPHICAL SKETCH

The author, Alfred Konrad Garin Aquino, was born on January 13, 1997 in Buenlag West, Mangaldan, Pangasinan as the eldest of Mario J. Aquino and Melchora E. Garin – Aquino. Mostly, he spent his childhood days with his grandmother because his parents worked as Overseas Foreign Worker (OFW).

He took his primary education at the United Methodist Church, Cinderella School where he participated in Student Organization and was elected as a student representative when he was in grade five and was awarded Athlete of the year on his graduation in 2009. During his highschool days at Mangaldan National High School he was very active in sports, music, and songwriting.

He passed the entrance examination in Central Luzon State University in May 2013 and was admitted in the Bachelor of Science in Agriculture curriculum. He chose Soil Science as his major field with specialization in Soil Biology. He joined the Soil Science Society which helped him improved his physical, mental and social skills.

Apart from being an agriculturist, the author would like to fulfill his main achievement in his life i.e., to serve not only for the betterment of his family but also to serve for the betterment of his beloved country.

ALFRED KONRAD G. AQUINO

ACKNOWLEDGEMENT

It does not matter what comes up. What matters is who you are, the one perceiving it.
- Mooji

I, the author of this book, humbly thank our Almighty God for giving the wisdom, knowledge, perseverance and strength to finish my manuscript and for bringing the following persons who helped me out for this achievement.

To my adviser, Prof. Florida C. Garcia, for the most untiring support, sharing her knowledge and ideas for the betterment of my study. Despite of her busy works, still she managed to have time to discuss and explain my thesis; to my critic, Dr. Purisima P. Juico, for accompanying me to my laboratory analyses and finding expert people who assisted in order to finish my DNA analyses; to our professor in coloqiuim, Dr. Apolinario L. Domingo, for giving such a valuable recommendation and suggestion for the improvement of my data and results.

To Dr. Federico O. Perez, Dr. Ariel G. Mactal and Prof. Fernan T. Fiegalan, for bringing out the greatest idea I ever had. Nourishing what the world of soil science is all about.

Ma'am Sheryl J. Marcha from Crop Protection Department, for giving her time in sterilizing the glassware and media needed for the isolation and for providing for the chemical and textural analyses of my soil sample.

Dr. Jerwin R. Undan from the College of Arts and Sciences, for providing the equipment and reagents needed for the DNA analysis of my rhizobial isolates.

To my brothers and sisters in Soil Science Society, thank you for the countless happiness, laughters, and tears, the friendship you had given to me and a chance to be part of your life.

Marita E. Garin, my grandmother, for the trust, love, patience and sustaining my financial needs to achieve my goals in life. Also to my parents, Mario J. Aquino and Melchora E. Garin, for being at my side in times of my difficulties. To my dearest uncle, Uncle Iran, for the unwavering help during the collection of my 30 soil samples needed for my thesis.

I would like to express my deepest thanks and sincere apreciation to Sir James Espiritu for giving me pieces of advice to strive more in life and for acccepting me not only as a resident but as a son in Agriculture Dorm.

To my friend, Paolo Adrian Ildefonso, for his assistance in watering my plants during weekends and for the the unwavering help and support to finish my manuscript when am about to give up my thesis during the toughest times.

To my DAYAMI family, Don, Venus, Bethel, Joy and Alma, for the wittiest experiences and joyful moments.

And lastly, to my beloved "Mahal", Jolina C. Dela Cruz, I will be forever grateful for having you in my life. Thank you for the love, comfort, trust, smile, laughter, tears, support and also the arguments we had not to be break our relationship but to improve and become mature enough to withstand the next difficulties we will ever have.

It is my pleasure to acknowledge my deepest thanks and gratitude to these people in my life.

TO GOD BE THE GLORY!

ALFRED KONRAD GARIN AQUINO

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

ISOLATION AND CHARACTERIZATION OF MUNGBEAN RHIZOBIA GROWN FROM SELECTED SOILS IN WESTERN PANGASINAN[1/]

by

ALFRED KONRAD GARIN AQUINO

ABSTRACT

This study was conducted to isolate and characterize rhizobia grown from selected soils of Western Pangasinan. Mungbean rhizobium was evaluated to understand their ecology and relatedness from one area to another. Twenty four isolates were tested for their morphological characteristics in YMA with Congo red and YMA with BTB. Rhizobium isolates were characterized as faintly pink to white, circular in shape with colony size of 0.5 mm to 3 mm diameter, opaque and translucent.

Sixteen out of 24 isolates were fast-growing and acid-producing bacteria classified as *Rhizobium* while five isolates were slow-growing and alkali-producing bacteria classified as *Bradyrhizobium.* All rhizobial isolates are Gram negative short rods. Out of 24 isolates, 3 were amplified under the gel documentation map. LI1 from Lingayen 1, BAY1 from Bayambang 1 and MAB1 from Mabini1. LI1 is *Bradyrhizobium* while BAY1 and MAB1 are both *Rhizobium.*

Correlation analysis between pH and nodulation of mungbean showed that there is a substantial relationship while EC, OM, soil texture and nodulation of mungbean do not have relationship.

Sixty-seven percent of the soils grown to mungbean in the 10 municipalities of Western Pangasinan are dominated by *Rhizobium* bacteria. Mungbean can be nodulated both by *Rhizobium* and *Bradyrhizobium.*

[1/]An undergraduate thesis manuscript presented in partial fulfillment of the requirements for graduation with the degree of Bachelor of Science in Agriculture major in Soil Science under the supervision of Prof. Florida C. Garcia with Research Contribution No. CA-05-17-0001.

LITERATURE CITED

AL FALIH, A.M.K. 2002. Factors affecting the efficiency of symbiotic nitrogen fixation by *Rhizobium*, Pakistan Journal of Biological Sciences, **5**:11, 1277-1293.

ALLEN E.K., and O.N. ALLEN. 1950. Bact. Revs., **14**:273 retrieved from http://himedialabs.com/TD/M721.pdf on August 29, 2016.

ANDREW, C. 1978. Legumes and Acid Soils. In; Dõbereiner, J., Burris, R.H., Hollaender, A.,Franco, A.A., Neyra, C.A. and Scott, D.B., Eds., *Limitations and Potentials for Biological Nitrogen Fixation in the Tropics*, Springer, New York, 135-160. Retrieved from http://dx.doi.org/10.1007/978-94-011-1088 4_13 on August 15, 2016.

BELNAP, J. 2001. Factors influencing nitrogen fixation and nitrogen release in biological soil crusts. In: *Biological Soil Crusts: Structure, Function and Management* (Belnap, J. and Lange, o. L. Eds.) Springer-Verlag Berlin Heidelberg, Germany, pp. 241-261. Retrieved from http://docsdrive.com/pdfs/ansinet/pjbs/2002/1277-1293.pdfon February 10, 2016.

BRUCKER, M, Z. 2012. Gram Staining. Retrieved from http://serc.carleton.edu/microbelife/research_methods/microscopy/gramstain.html on August 22, 2016.

BOSSHARD, P.P., R. ZBINDEN, S. ABELS, B. BODDINGHAUS, M. ALTWEGG, and E.C. BOTTGER. 2006. 16S rRNA gene sequencing versus the API 20 NE system and the Vitek 2 ID-GNB card for identification of nonfermenting gram-negative bacteria in the clinical laboratory.J. Clin. Microbiol. **44**:1359 – 1366.

CORREA, O.S., and A.J. BARNEIX. 1997. Cellular mechanisms of pH tolerance in *Rhizobium loti*. World J Microbiol Biotechnol, **13**:153 – 157.

CTAHR, (2000). Biological Nitrogen Fixation. *Nature's Partnership for Sustainable Agricultural Production*. Retrieved from http://www.ctahr.hawaii.edu/oc/freepubs/pdf/pnm13.pdf on August 13, 2016.

EAGLESHAM, A.R.J., and A. AYANABA. 1984. Tropical Stress Ecology of Rhizobia, RootNodulation and Legumes Fixation. In: Subba Rao, N.S., Ed., *Current Developments in Biological Nitrogen Fixation*, Edward Arnold Ltd., London,1-35.

ELBOUTAHIRI, N.I., S.M. THAMI-ALAMI, and UDUPA. 2010. Phenotypic and genetic diversity in *Sinorhizobiummeliloti* and *S. medicae* from drought and salt

affected regions of Morocco, Institut National de la Recherche Agronomique (INRA).

FLYNN, R., and J. IDOWU. 2015. Nitrogen Fixation by Legumes. Retrieved from http://aces.nmsu.edu/pubs/_a/A129.pdf on August 14, 2016.

GWATA, E.T., S.D. WOFFORD, J.K. BOOTE and H. MUSHORIWA. 2003. Determination of effective nodulation in early juvenile soybean plants for genetic and biotechnology studies. *Afr. J. Biotechnol.*, **2**, 417-420.

GAURI, SINGH A.K., R.P. BHATT, S. PANT, M.K. BEDI, and A. NAGLO. 2011. Characterization of *Rhizobium* isolated from root nodules of *Trifolium alexandrinum*. Journal of Agricultural Technology 7(6): 1705 1723.

JANDA, J.M., and S.L. ABBOTT. 2007. 16S rRNA Gene Sequencing for Bacterial Identification in the Diagnostic Laboratory: Pluses, Periles, and Pitfalls. *Journal of Clinical Microbiology, Sept. 2007*, p. 2761-2764

KRYNDUSKIN, D.S., L.M. ALEXANDROV, M.D. TER-AVANESYAN, and V.V. KUSHNIROV. 2003.Yeast PSI+ prion aggregates are formed by small Sup35polymers fragmented by Hsp10. *Journal of Biological Chemistry*.278 (49): 49636.

LAMB, J.A., F.G. FERNANDEZ, and D.E. KAISER. 2014. Understanding Nitrogen in Soils. *Extension Specialists in Nutrient Management*. Retrieved from http://www.extension.umn.edu/agriculture/nutrient-management/nitrogen/understanding nitrogen-in-soils/docs/AG-FO-3770-B.pdf on September 10, 2016.

LEDGARD, S., and K. STEELE. 1992. Biological Nitrogen Fixation in Mixed Legume/Grass Pastures. *Plant and Soil*, 141, 137-153. Retrieved from http://dx.doi.org/10.1007/BF00011314 on August 15, 2016.

LINDERMANN, W.C., and C.R. GLOVER. 1990. Nitrogen Fixation by Legumes. Guide A-129.

LIU, C., and J.D. MURRAY. 2016. The Role of Flavonoids in Nodulation Host-Range Specificity: An Update. Retrieved from file:///C:/Users/User/Downloads/plants-05 00033.pdf on August 23, 2016.

MARUEKARAJTINPLENG S., W. HOMHAUL, and C.N. KANJANA. 2012. Presence of natural variants of *Bradyrhizobium elkanii* and *Bradyrhizobium japonicum* and detection of *Bradyrhizobium yuanmingense* in Phitsanulok province, Thailand. ScienceAsia 38 (2012): 24-25.

MICHELS, J., C. VERRETH, and J. VANDERLEYDEN. 1994. Effects of temperature stress on bean nodulation *Rhizobium* strains. Appl Environ Microbiol, **60**:1206 – 1212.

NISTE, M., R. VIDICAN, L. ROTAR, and P. ROTAR. 2013. Stress Factors Affecting Symbiosis Activity and Nitrogen Fixation by *Rhizobium* Cultured *in vitro*. 3 – 5:400372. Retrieved from http://journals.usamvcluj.ro/index.php/promediu/article/viewFile/9100/7751 on August 22, 2016.

ÖĞÜTÇÜ H., C. KASIMOĞLU, and E. ELKOCA. 2010. Effects of rhizobium strains isolated from wild chickpeas on the growth and symbiotic performanceof chickpeas (*Cicer arietinum* L.) under salt stress, Turk J Agric. For, **34**, 361 – 371.

PONGSILIP, N. 2012. Genotypic diversity of Rhizobia Assessed by Polymerase Chain Reaction (PCR) fingerprinting. *Department of Microbiology, Faculty of Science, Silpakorn University, Nakhon Pathom, Thailand.*

RAO D.L.N., K.E. GILLER, A.R. YEO, and T.J. FLOWERS. 2002. The effects of salinity and sodicity upon nodulation and nitrogen fixation in chickpea (*Cicer arietinum*). Retrieved from http://aob.oxfordjournals.org/content/89/5/563.long on August 22, 2016.

SAMBROOK, J., and D.W. RUSSEL. 2001. Molecular Cloning: A Laboratory Manuel 3rd Ed. Cold Spring Harbor Laboratory Press. Cold Spring Harbor, NY.

SHERWOOD, M. 1972. Inhibition of *Rhizobium trifolii* by Yeast Extracts or Glycine is prevented by Calcium. *Agricultural Institute, Johnstown Castle, Wexford, Ireland and Biochemistry Department, Unviversity College, Dublin, Ireland*, **71**, 351-358.

SIMON Z., M. KELVIN, E. AMARE, and P.A. NDAKIDEMI. 2014. Isolation and Characterization of Nitrogen Fixing Bacteria from Cultivated and Uncultivated Soils of Northern Tanzania. American Journal of Plant Science 5: 4050 4067.

SØRENSEN, J., and A. SESSITSCH. 2007. Plant-associated bacteria—Lifestyle and molecular interactions. In J.D. van Elsas, J.K. Jansson, and J.T. Trevors (Eds.), Modern Soil Microbiology, 2nd ed. (pp. 211–236). Boca Raton, FL: CRC Press, Taylor and Francis Group.

SUBBA RAO. S. 1977. Soil Microorganisms and Plant Growth, Oxford and IBH Publishing Co. Retrieved from http://himedialabs .com/TD/M721.pdf. on August 29,2016.

WALMORTH, J. 2013. Nitrogen in Soil and the Environment. Retrieved from http://extension.arizona.edu/sites/extension.arizona.edu/files/pubs/az1591.pdf on September 10, 2016.

WWW.BIBLIO.TELUQ.CA. 2002. Rhizobium, Root Nodules & Nitrogen Fixation. http://biblio.teluq.ca/LinkClick.aspx?fileticket=Zt582qIwcMI5ljHgoneZIw%3D%3D&t bid=40831&language=fr-CA.

WWW.FAO.ORG. 1989. Technical Paper 2: Biological Nitrogen Fixation. Retrieved fromhttp://www.fao.org/Wairdocs/ILRI/x5546E/x5546e05.htm#2.3specificity_and_eff ective ess on August 10, 2016.

WWW.MOLECULAR.ROCHE.COM. 1996-2016. What is PCR. Retrieved from https://molecular.roche.com/innovation/pcr/what-is-pcr/ on August 28, 2016.

WWW.UVM.EDU, Comparison of phenotypic and genotypic techniques for identifcation of unsual aerobic pathogenic gram-negative bacilli Retrieved from https://www.uvm.edu/~gdrusche/Classes/GEOL%20295%20%20Geomicrobiology/Lectures/Lecture3.ppt on August 31, 2016.

WWW.VLAB.AMRITA.EDU. 2013. Soil Analysis-Determination of Available Organic Carbon content in the Soil. Retrieve from http://vlab.amrita.edu/?sub=2&brch=294&sim=1552&cnt=1 on August 14, 2016.

YAMAGUCHI T., and E. BLUMWALD. 2005. Developing salt-tolerant crop plants: Challenges and opportunities, Trends in Plant Science, 10:12, 615 – 620.

ZAHRAN, H.H. 1999. Rhizobium-legume symbiosis and nitrogen fixation under severe conditions and in an arid climate. Retrieved from http://www.ncbi.nlm.nih.gov./pubmed/10585971 on June 10, 2017.