ISOLATION OF EIGHT BACTERIAL STRAINS FROM RHIZOSPHERIC SOIL OF PEANUT FIELDS

May Zin Moe[1], Zar Zar Yin[2] and Soe Soe Aung[3]

Abstract

The bacterial strains were isolated from the rhizospheric soil of peanut fields. The samples were collected from Shan Ka Lay Kyun village, Amarapura Township, Mandalay Region during August 2016. This experiment was carried out at the Microbiology Laboratory, Department of Botany, University of Mandalay from August 2016 to February 2017. The bacterial strains were isolated by using the King's B medium. The bacterial strains MZ 1, MZ 2, MZ 3, MZ 4, MZ 5, MZ 6, MZ 7, MZ 8 were isolated. These bacterial strains were identified based on their colony morphology and biochemical activities such as catalase test, oxidase test, sugar fermentation test (sucrose, lactose), methyl red (MR) test, voges-proskauer (VP) test, lysine decarboxylase test, citrate utilization test, nitrate reduction test, urease test, triple sugar iron test, starch hydrolysis test, oxygen requirement and motility test. According to the results, the isolated bacterial strains, MZ 1- MZ 8 were confirmed as *Pseudomonas* sp. The colony and cell morphology of eight bacterial strains were described and presented with photomicrographs.

Keywords: rhizospheric soil of peanut, isolation, identification of bacterial strains, *Pseudomonas* sp.

Introduction

Soil microorganisms, such as bacteria and fungi, control ecosystem functioning through decomposition and nutrient cycling and may serve as indicators of land-use change and ecosystem health (Balser *et al*., 2010). The rhizosphere area includes the soil connected to the plant roots and often extends a few millimeters of the root surface, being an important environment for the plant and microorganism interactions (Lynch, 1990 and Gray and Smith, 2005 as cited in Souza *et al*., 2015), because plant roots release a wide range of compounds involved in attracting organisms which may be beneficial, neutral or detrimental to plants (Lynch, 1990 and Badri and Vivanco, 2009 as cited in Souza *et al*., 2015).

[1.] Assistant Lecturer, Department of Botany, University of Mandalay
[2.] Associate Professor, Department of Botany, Pathein University
[3.] Professor, Department of Botany, University of Mandalay

The plant growth-promoting bacteria (PGPB) belong to a beneficial and heterogeneous group of microorganisms that can be found in the rhizosphere, on the root surface or associated to it, and are capable of enhancing the growth of plants and protecting them from diseases and abiotic stresses (Dimkpa *et al.,* 2009, Grover *et al.,* 2011 and Glick, 2012 as cited in Souza *et al*., 2015). Certain plant growth promoting microorganisms could enhance defensive activity and stimulate plant resistance against soil borne pathogens (Whipps *et al*., 2001 as cited in Ardebili *et al.,* 2011). Both rhizosphere and rhizoplane comprised *Bacillus, Enterobacter* and *Pseudomonas* can be applied as PGPB to improve and enhance growth in arid soils. Isolated bacteria from soils were found to exhibit capabilities in fix atmospheric nitrogen, produce ammonia, indoleacetic acid (IAA), siderophores, solubilize phosphate and zinc (El-Sayed and El-Naggar, 2014).

Beneficial microorganisms that improve plant health through the enhancement of plant resistance and tolerance against biotic stresses include bacteria, such as *Pseudomonas* sp. or *Bacillus* sp. and fungi such as *Trichoderma* sp., *Gliocladium* sp. or mycorrhizal fungi (Pozo *et al.,* 2002 as cited in Ardebili *et al.,* 2011). Many rhizosphere colonizing bacteria, including *Azotobacter*, *Azospirillum*, *Bacillus*, *Clostridium*, and *Pseudomonas*, typically produce substances that stimulate plant growth or inhibit root pathogens (Vázquez *et al*., 2000 as cited in Ardebili *et al.,* 2011).

Therefore, the present study was carried out the isolation and identification of bacterial strains from the rhizospheric soil of peanut growing areas. The aim and objectives of this study were to isolate the bacterial strains from rhizospheric soil of peanut and to study their colony morphology characters, gram straining, microscopical characters and biochemical activities.

Materials and Methods

The soil samples were randomly collected from rhizospheric soil of peanut fields in Shan Ka Lay Kyun Village, Amarapura Township, Mandalay Region during August 2016. The soil samples were collected from these regions (Figure 1). The study was carried out at the Microbiology Laboratory, at the Department of Botany, University of Mandalay from August, 2016 to February, 2017.

Serial dilutions of soil samples, plating and streaking techniques were used to isolate the microorganisms from soil according to Salle (1948); Collins (1964) and Pelezer and Chan (1972).

The identification of isolated bacterial strains were carried out using their colony morphology, gram staining methods (Dubey and Maheshwari, 2002), and biochemical activities which include Catalase test (Dickey and Kelman, 1988), Oxidase test (Dickey and Kelman, 1988), Sugar fermentation test (sucrose, lactose) (Atlas, 1993), Methyl red test (Aneja, 1996), Voges-Proskauer-VP test (Cruickshank, 1963), Lysine decarboxylase test (Downes, and/to 2001), Citrate utilization test (Atlas, 1993), Nitrate reduction test (Dickey and Kelman, 1988), Urease test (Woodland, 2004), Triple sugar iron test (Woodland, 2004), Starch hydrolysis test (Aneja, 1996), Oxygen requirement (aerobic/anaerobic) (Prescott, 2002)and motility test (Tittsler and Sandholzer, 1936).

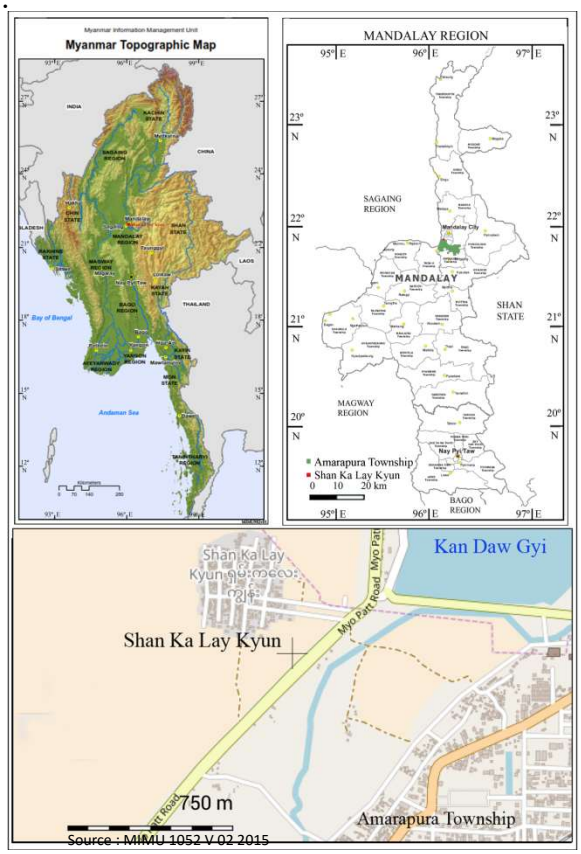

Figure. 1 Location Map of Specimen Collection Site in Shan Ka Lay Kyun village, Amarapura Township

Results

The total of 8 bacterial strains such as MZ 1, MZ 2, MZ 3, MZ 4, MZ 5, MZ 6, MZ 7 and MZ 8, were isolated from the rhizospheric peanut soil. The colonies morphology of those isolated strains MZ 1-MZ 8 were small, moderate, large in sizes; circular, irregular, entire in margins; cream, white, pale green and pale yellow in color; raised and flat in elevation and form; shiny and dull in pigments on agar. Those bacterial strains were short-rot and rod in their cell morphologies, facultative and aerobic, positive in motality. The results of their colony morphology, cell morphology and biochemical activities were shown in Table 1-3 and Figure 2-22.

Table 1. Colony characteristics of eight strains

Strains	Size of Colony	Margin	Color	Elevation and Form	Apperance
MZ 1	small	circular	cream	raised	shiny
MZ 2	moderate	circular	white	raised	shiny
MZ 3	small	circular	white	flat	shiny
MZ 4	large	irregular	pale green	raised	dull
MZ 5	small	entire	pale green	raised	shiny
MZ 6	small	irregular	cream	raised	shiny
MZ 7	small	entire	white	raised	shiny
MZ 8	moderate	entire	pale yellow	raised	shiny

Table 2. Cell morphology and physical characteristics of eight strains

Strains	Gram Staining	Cell Shape	Physical Characteristics
MZ 1	–	rod	aerobic
MZ 2	–	short rod	aerobic
MZ 3	–	rod	aerobic
MZ 4	–	rod	aerobic
MZ 5	–	short rod	aerobic
MZ 6	–	rod	aerobic
MZ 7	–	rod	aerobic
MZ 8	–	short rod	facultative

negative reaction = –

Table 3. Biochemical activities of eight strains

No.	Biochemical activities	Strains							
		MZ 1	MZ 2	MZ 3	MZ 4	MZ 5	MZ 6	MZ 7	MZ 8
1	Catalase	+	+	+	+	+	+	+	+
2	Oxidase	+	+	+	+	+	+	+	+
3	Sugar Fermentation (lactose)	+	+	+	+	–	–	–	+
4	Sugar Fermentation (sucrose)	+	+	+	+	+	+	–	+
5	Methyl Red (MR)	–	–	–	–	–	–	–	–
6	Voges-Proskauer (VP-Test)	–	–	–	–	–	–	–	–
7	Lysine	+	–	+	–	–	+	+	–
8	Citrate	+	+	+	+	+	+	+	+
9	Nitrate	+	–	+	–	–	+	+	+
10	Urease	+	+	–	+	+	–	–	–
11	Triple sugar								
	(i) Fermentation	+	+	+	+	+	+	+	+
	(ii) H_2S	–	–	–	–	+	–	–	–
	(iii) Gas	–	+	–	–	–	–	–	+
12	Starch Hydrolysis	+	–	+	–	–	–	–	–
13	Motality	+	+	+	+	+	+	+	+

positive reaction = +, negative reaction = –

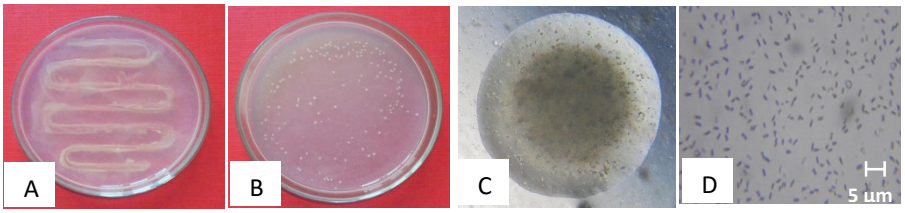

Figure 2. Colony characteristics and cell morphology of MZ 1

A. Colonies on streaks plate, B. Colonies, C. Single colony,

D. Photomicrograph of cells

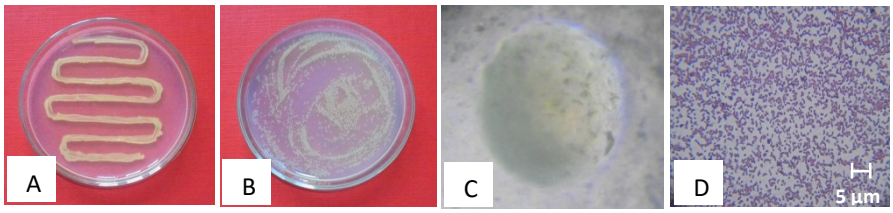

Figure 3. Colony characteristics and cell morphology of MZ 2
A. Colonies on streaks plate, B. Colonies, C. Single colony,
D. Photomicrograph of cells

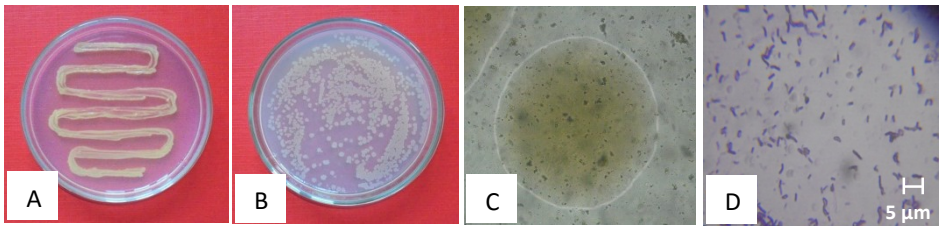

Figure 4. Colony characteristics and cell morphology of MZ 3
A. Colonies on streaks plate, B. Colonies, C. Single colony,
D. Photomicrograph of cells

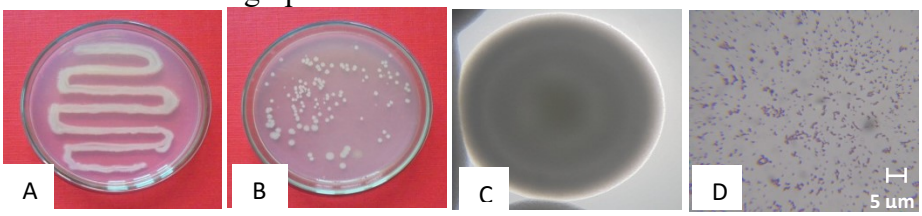

Figure 5. Colony characteristics and cell morphology of MZ 4
A. Colonies on streaks plate, B. Colonies, C. Single colony,
D. Photomicrograph of cells

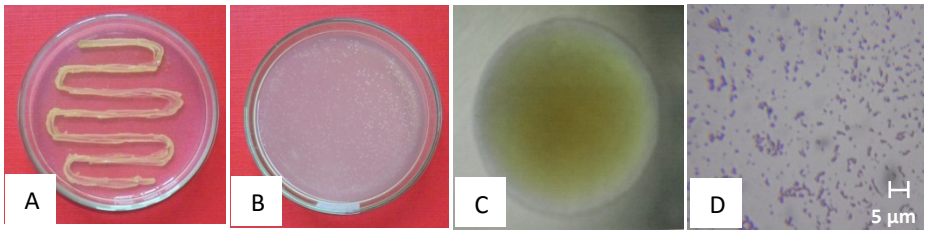

Figure 6. Colony characteristics and cell morphology of MZ 5
A. Colonies on streaks plate, B. Colonies, C. Single colony,
D. Photomicrograph of cells

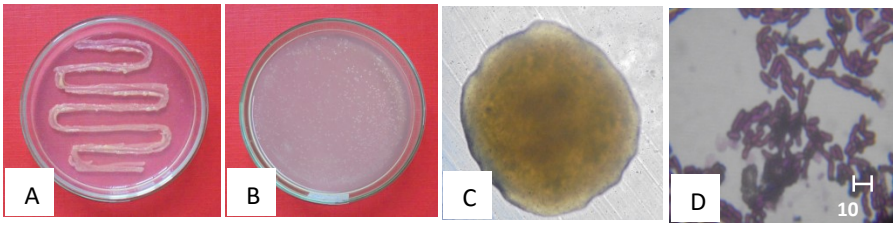

Figure 7. Colony characteristics and cell morphology of MZ 6
A. Colonies on streaks plate, B. Colonies, C. Single colony,
D. Photomicrograph of cells

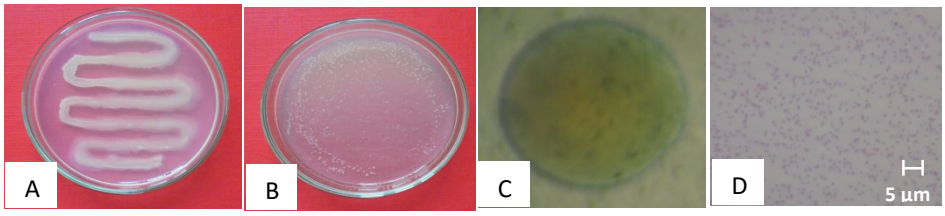

Figure 8. Colony characteristics and cell morphology of MZ 7
A. Colonies on streaks plate, B. Colonies, C. Single colony,
D. Photomicrograph of cells

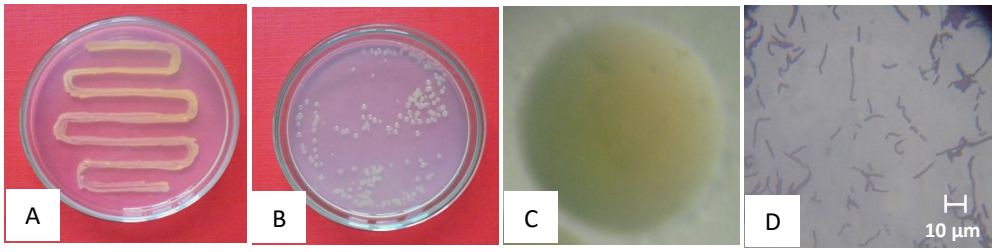

Figure 9. Colony characteristics and cell morphology of MZ 8
A. Colonies on streaks plate, B. Colonies, C. Single colony,
D. Photomicrograph of cells

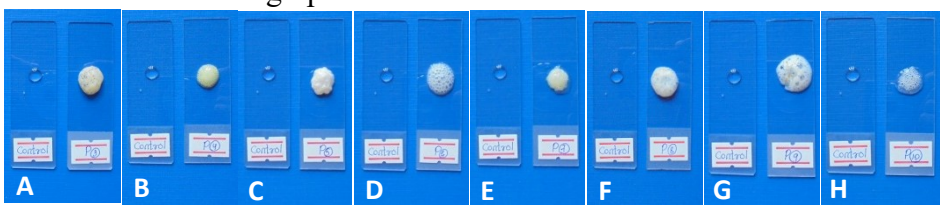

Figure 10. Catalase test A. MZ 1 (Positive), B. MZ 2 (Positive), C. MZ 3 (Positive), D. MZ 4 (Positive), E. MZ 5 (Positive), F. MZ 6 (Positive), G. MZ 7 (Positive), H. MZ 8 (Positive).

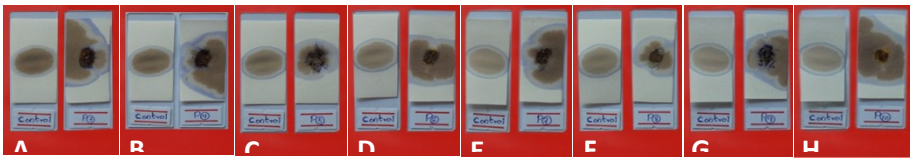

Figure 11. Oxidase test A. MZ 1 (Positive), B. MZ 2 (Positive), C. MZ 3 (Positive), D. MZ 4 (Positive), E. MZ 5 (Positive), F. MZ 6 (Positive), G. MZ 7 (Positive), H. MZ 8 (Positive)

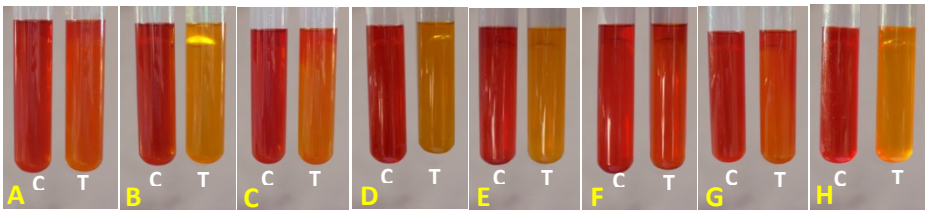

Figure 12. Sugar fermentation test (lactose) A. MZ 1 (Positive), B. MZ 2 (Positive), C. MZ 3 (Positive), D. MZ 4 (Positive), E. MZ 5 (Negative), F. MZ 6 (Negative), G. MZ 7 (Negative), H. MZ 8 (Positive). C = control, T = treatment.

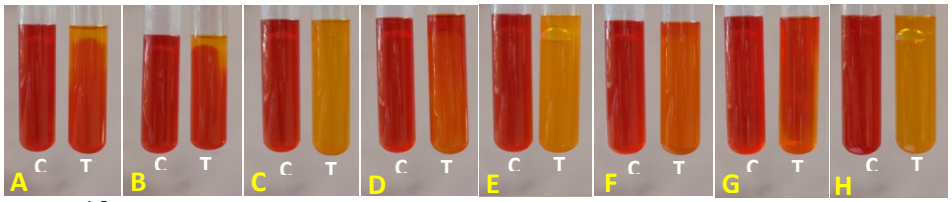

Figure 13. Sugar fermentation test (sucrose) A. MZ 1 (Positive), B. MZ 2 (Positive), C. MZ 3 (Positive), D. MZ 4 (Positive), E. MZ 5 (Positive), F. MZ 6 (Positive), G. MZ 7 (Negative), H. MZ 8 (Positive). C = control, T = treatment.

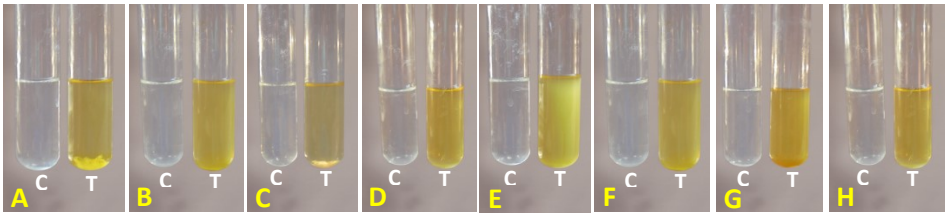

Figure 14. Methyl red (MR) test A. MZ 1 (Negative), B. MZ 2 (Negative), C. MZ 3 (Negative), D. MZ 4 (Negative), E. MZ 5 (Negative), F. MZ 6 (Negative), G. MZ 7 (Negative), H. MZ 8 (Negative).C = control, T = treatment.

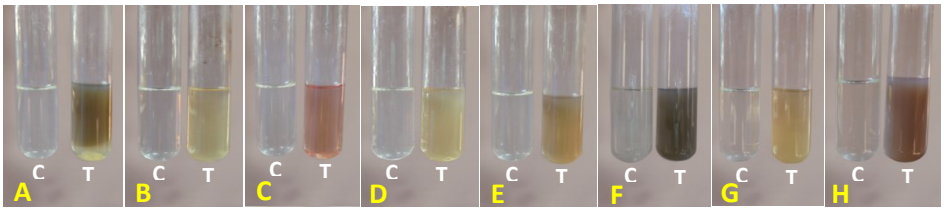

Figure 15. Voges-Proskauer (VP) test A. MZ 1 (Negative), B. MZ 2 (Negative), C. MZ 3 (Negative), D. MZ 4 (Negative), E. MZ 5 (Negative), F. MZ 6 (Negative), G. MZ 7 (Negative), H. MZ 8 (Negative). C = control, T = treatment.

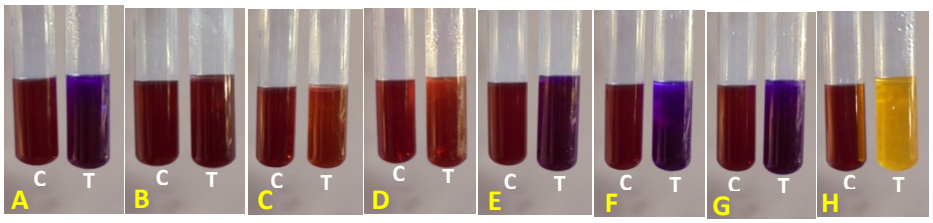

Figure 16. Lysine test A. MZ 1 (Positive), B. MZ 2 (Negative), C. MZ 3 (Positive), D. MZ 4 (Negative), E. MZ 5 (Negative), F. MZ 6 (Positive), G. MZ 7 (Positive), H. MZ 8 (Negative). C = control, T = treatment.

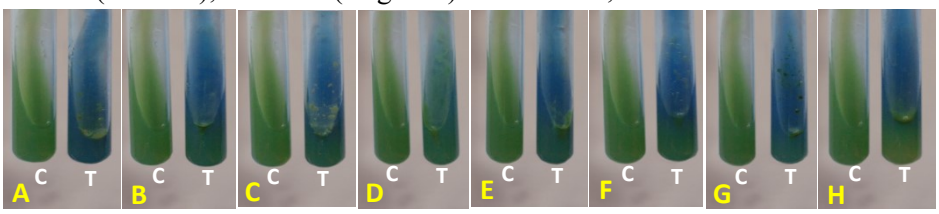

Figure 17. Citrate test A. MZ 1 (Positive), B. MZ 2 (Positive), C. MZ 3 (Positive), D. MZ 4 (Positive), E. MZ 5 (Positive), F. MZ 6 (Positive), G. MZ 7 (Positive), H. MZ 8 (Positive). C = control, T = treatment.

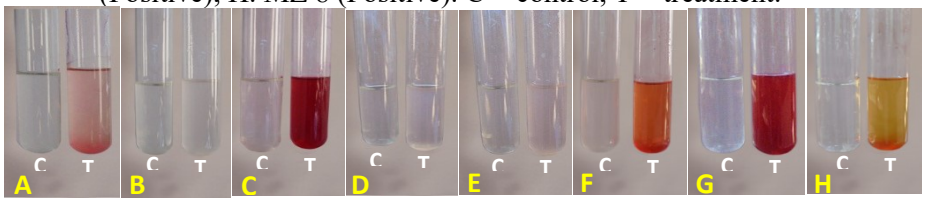

Figure 18. Nitrate test A. MZ 1 (Positive), B. MZ 2 (Negative), C. MZ 3 (Positive), D. MZ 4 (Negative), E. MZ 5 (Negative), F. MZ 6 (Positive), G. MZ 7 (Positive), H. MZ 8 (Positive). C = control, T = treatment.

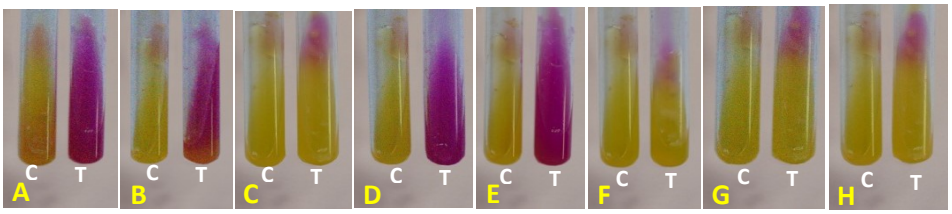

Figure 19. Urease test A. MZ 1 (Positive), B. MZ 2 (Positive), C. MZ 3 (Negative), D. MZ 4 (Positive), E. MZ 5 (Positive), F. MZ 6 (Negative), G. MZ 7 (Negative), H. MZ 8 (Negative). C = control, T = treatment.

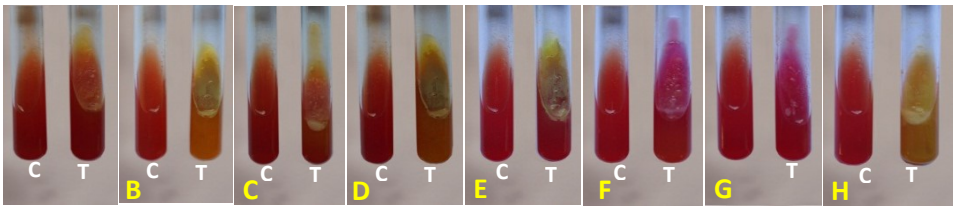

Figure 20. Triple sugar test A. MZ 1 (Negative), B. MZ 2 (Positive), C. MZ 3 (Positive), D. MZ 4 (Positive), E. MZ 5 (Positive), F. MZ6 (Positive), G. MZ 7 (Negative), H. MZ 8 (Positive). C = control, T = treatment.

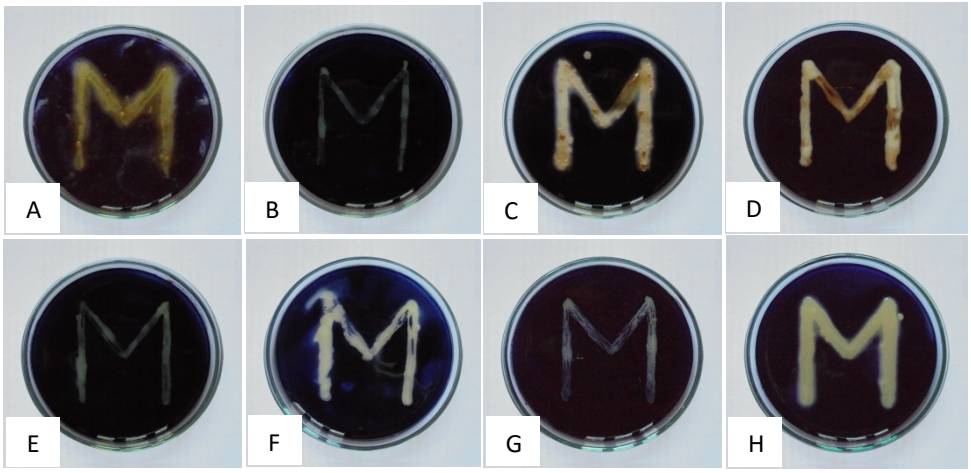

Figure 21. Starch hydrolysis test [A. MZ 1 (Positive), B. MZ 2 (Negative), C. MZ 3 (Positive), D. MZ 4 (Negative), E. MZ 5 (Negative), F. MZ 6 (Negative), G. MZ 7 (Negative), H. MZ 8 (Negative)].

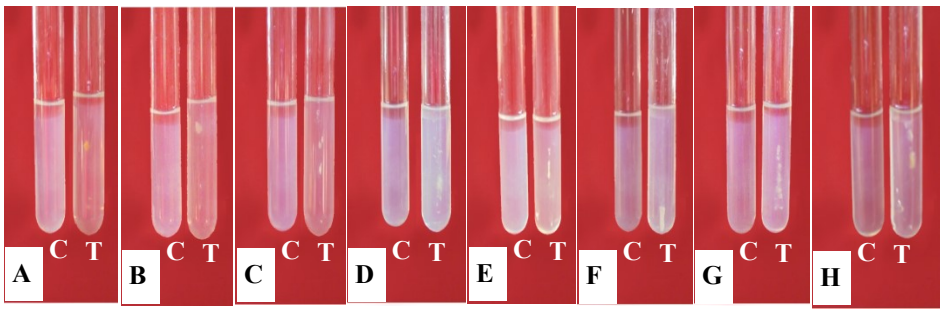

Figure 22. Motality tests. A. MZ 1 (Positive), B. MZ 2 (Positive), C. MZ 3 (Positive), D. MZ 4 (Positive), E. MZ 5 (Positive), F. MZ 6 (Positive), G. MZ 7 (Positive), H. MZ 8 (Positive). C = control, T = treatment.

Discussion and Conclusion

Microorganisms play an important role on the nutritional chains that are an important part of the biological balance in the life in planet. Microorganisms can be used to determine the bioavailability of a given chemical compounds in soil (Bating *et al*., 2008).

The present study, the rhizospheric soil samples were randomly collected from the peanut fields of Shan Ka Lay Kyun village, Amarapura Township, Mandalay Region during August 2016. The study was carried out at Microbiology Laboratory of Botany Department, University of Mandalay from August 2016 to February 2017.

The present findings showed that total of eight bacterial strains were isolated and identified from the rhizospheric peanut soils. The isolated strains were designated as MZ 1, MZ 2, MZ 3, MZ 4, MZ 5, MZ 6, MZ 7, MZ 8. In order to identify the isolated strains, the colony morphology, gram strains, microscopical characters and biochemical tests were carried out during the study. As the results, the colony sizes of MZ 1 - MZ 8 were small, moderate and large. Their margins were circular, irregular and entire. Their colony color were cream, white, pale-green and pale yellow. The elevation of these strains was raised and flatted. The colony's pigment was shiny and dull on agar medium. All of the isolated strains MZ 1 to MZ 8 in their cell morphology were gram-negative, rod and short-rod, aerobic, motile, catalase positive; oxidase positive. In addition, lactic acids were produced from sucrose and lactose, negative results in methyl red (MR) and (VP) tests, positive in the

citrate and nitrate reactions. However, H_2S gas was not produced except in MZ 5. All the isolated strains showed negative in starch hydrolysis except in MZ 1 and MZ 3.Those results were based on the identification of colony morphology, microscopical characters and biochemical reactions from the isolated strains which were more or less the same characters of *Pseudomonas* spp. These findings were also in agreement with Soesantoetal. (2011) who reported that *Pseudomonas* was greenish yellow colony, smooth edges, convex surfaces and fluorescent under UV light on King's B agar medium. These results were the same with the descriptions of Buchanan and Gibbons (1974). Therefore, these isolated strains MZ 1 - MZ 8 were identified as the genus, *Pseudomonas.*

As the results, it would be concluded that the present findings of those isolated bacterial strains MZ 1 - MZ 8 can be noted as the *Pseudomonas* bacterial strains. Those bacterial strains would be isolated from the rhizopheric soil of peanut fields and confirmed as *Pseudomonas* spp. However, further study should be undertaken for the antimicrobial activities and biocontrol agents by using the effective bacterial strains which can be isolated from different rhizospheric soils.

Acknowledgements

I would like to acknowledge to the following persons who have supported for this research work: Dr. Nu Nu Yee, Professor and Head of Department of Botany, University of Mandalay for her invaluable advice and encouragement, Dr. Soe Myint Aye, Professor from Department of Botany, University of Mandalay for his critical reviews and suggestions and Dr. Win Win Khaing, Lecturer, Department of Botany, University of Mandalay for great helps and suggestions.

References

Aneja, K.R. (1996). Experiments in Microbiology, Plant Pathology, Tissue Culture and mushroom cultivation. Wishwa Prakashan New Age Interational (P) Limited. New Delhi.

Ardebili, Z.O., N.O. Ardebili and S.M.M. Hamdi. (2011). Physiological effects of *Pseudomonas fluorescens* CHAO on tomato (*Lycopersicon esculentum* Mill.) plants and its possible impact on *Fusarium oxysporum* f. sp. *lycopersici*. AJCS 5(12): 1631-1638. ISSN.

Atlas, R.M. (1993). Microbiological media. Boca Raton Ann Arbor, London Tokyo. Australia. Bacteriology. 8th Edition. Williams Wilkinson Co. Ltd. Baltimore. p. 244.

Balser, T.C., D. Wixon, L.K. Moritz and L. Lipps. (2010). The Microbiology of natural soils. Chapter 2. G. R. Dixon and E. L. Tilston (eds.), Soil Microbiology and Sustainable Crop Production DOI 10, Springer Science Business Media B.V.

Bating, A.E., E.A. Aly, A.A. Khaled and K.A. Anel. (2008). Isolation, characterization and application of bacterial population from agricultural soil. Sohag Province, Egypt. Vol. 2. Malaysian Journal of Microbiology, (2), 45-50.

Bawa, I. (2016). Management strategies of Fusarium wilt disease of tomato incited by *Fusarium oxysporum* f. sp. lycopergici (sacc.). A review. International Journal of Advanced Academic Research, Sciences, Technology & Engineering. ISSN: 2488-9849. Vol.2, Issue 5.

Buchanan, R.E. and N.E. Gibbons. (1974). Bergey's manual of determinative Bacteriology. 8th Edition. Williams Wilkinson Co. Ltd. Baltimore. p. 244.

Collin, C. H. (1964). Microbiological methods.

Christerison, W.B. (1946). Urea decomposition as a means of differentiating *Proteus* and *Paracolon* cultures from each other and *Salmonella* and *Shigella*. J. Bact., p. 521-641.

Cruickshank, R., J.P. Guguid and R.H.A. Swain**. (**1963). Medical Microbiology. 11th Edition. The English Language Book Society and F. and S. Living Stone Ltd., London.

Dickey, R.S. and A. Kelman. (1988). Caratovora or soft rot group. In: Laboratory guide for identification of plant pathogenic bacteria 2nd Edition. (Ed. N.W. Schaad). Minnesota. p. 81-84.

Downes. F.P. and K. Ito. (2001). Compendium of methods for the microbiology examination of foods. 4th Edition. American Public Health Association, Washington, D.C.

Dubey, R.C. and D.K. Maheswari. 2002. Practical Microbiology. S. Chand & Co., New Delhi.

El-Sayed, W.S. & M.Y. El-Naggar. (2014). *In vitro* antagonistic activity, plant growth promoting traits and phylogenetic affiliation of rhizobacteria associated with wild plants grown in arid soil. Egypt. Microbial, 4; 5: 651.

Pelezar, M. J. & E. C. S. Chan. **(**1972). Exercises in microbiology. 3rd ed. McGraw. Hill Book Co., New York. Pennsylvania State University Pesticide Education and Assessment Program.

Prescott, H. (2002). Laboratory exercises in Microbiology. McGraw-Hill Companies.

Salle, A.J. (1948). Fundamental principles of Bacteriology. Mc. Graw Hill Book Co., Inc., New York.

Soesanto L., E. Mugiastuti and R.F. Rahayuniati. (2011). Morphological and physiological features of *Pseudomonas fluorescens*. Faculty of Agriculture, Jenderal Soedriman University, Purwokerto. p. 60.

Souza, R. De., A. Ambrosini and L.M.P. Passaglia. (2015). Plant growth-promoting bacteria as inoculants in agricultural soils. Genetics and Molecular Biology. 38, 4, 401-419.

Tittsler, R.P. and L.A. Sandholzer. (1936). The use of semi-solid agar for the detection of bacterial motility. J. Bacterial., 31: 576- 580.

Woodland, J. (2004). Bacteriology. NWFHS Laboratory Procedure Manual. Second Edition, Pinetop, Arizona.