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Keywords	Rhizospheric soil of peanut, isolation, identification of bacterial strains, *Bacillus* spp.
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Isolation and Identification of Eleven Bacterial Strains from Rhizospheric Soil of Peanut Fields

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

Abstract

The soil samples were randomly collected from the rhizospheric soil of peanut fields in Shangalay Kyun Village, Amarapura Township, Mandalay Region during August 2016. This study was carried out at Microbiology Laboratory of Botany Department, University of Mandalay from August 2016 to February 2017. In order to the isolation of bacterial strains, the ATCC 552 medium was used in this study. As the results, total of 8 bacterial strains (MZ 1 to MZ 11) were isolated and identified with their colony morphology, gram straining, microscopical characters and biochemical reactions. The colony morphology of isolated bacterial strains, MZ 1 to MZ 11 were large, moderate, small in their sizes and irregular, circular, and entire in margins. All isolated bacterial strains were gram positive. In facts, eleven isolated bacterial strains from MZ 1 to MZ 11 were identified and characterized as the genus, *Bacillus* spp.

Keywords: rhizospheric soil of peanut, isolation, identification of bacterial strains, *Bacillus* spp.

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 *Bacillu*s genus (Cohn 1872 as cited in Alina 2015) includes Gram positive, rod shaped bacterial cells of 0.3-2.2 to 1.2-7.0 μm, most of them motile, with peritrich flagella. These bacteria are chemoheterotrophes that can use various nutritional substrates. They can exhibit either fermentative or both respiratory and fermentative pathway to produce energy. As respiration, *Bacillus* species can be obligate aerobe or facultative anaerobe. The terminal electron acceptor is the molecular oxygen (O_2),

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replaced at some species by nitrate (NO_3) in special conditions (Hoffmann 2005 as cited in Alina 2015). The morphology and colony size are highly variable characteristics, depending on the environmental conditions. In the presence of specific nutrients, some species can produce pigments. Most species are widespread in nature. Many of the *Bacillus* sp. form resistant endospores, with no more than one endospore in the sporangia cell. The endospores can be distinguished from vegetative cells, as they are refractile and less colored, containing dipicolinic acid, 5 to 15% in dry weight. These endospores are dormant structures, non-reproductive, they can survive without nutrients and resist to extreme physical and chemical agents (Huang 2007 as cited in Alina 2015).

Bacillus genus is a heterogeneous taxon, with ubiquitous spread in nature. *Bacillus* species, such as *Bacillus cereus*, *B. megaterium*, *B. subtilis*, *B.circulans* and *B. brevis* group are widely exploited for biotechnological and industrial applications (Xu D *et al*. 2003 & Rooney *et al.* 2009 as cited in Alina 2015). Their beneficial traits for plant protection and growth promotion comprise the synthesis of broad-spectrum active metabolites, easily adaptation in various environmental conditions, benefic plantbacterial interaction and advantageous formulation process (Constantinescu *et al.* 2013 as cited in Alina 2015).

Therefore, the present study was carried out the isolation and identification of bacterial strains from the rhizospheric soil of peanut plants. The aim and objectives of this study were to isolate the bacterial strains from rhizospheric soil of peanut and to identify those bacterial strains based on their colony morphology, gram straining, microscopical characters and biochemical reactions.

Materials and Methods

The rhizospheric soils of peanut fields were randomly collected from Shangalay Kyun Village, Amarapura Township, Mandalay Region from August to December, 2016. This experiment was carried out at the Microbiology Laboratory, at the Department of Botany, University of Mandalay.

Isolation of the collected soil samples was done in laboratory as soon as possible after soil collection in fields. In order to isolate bacteria from soil samples, the soil samples were dried in air and ground, and finally kept in a clear flask.

Serial dilutions of fermented, plating and streaking techniques were used to isolate the microorganisms from soil according to Salle (1948); Collins (1965) 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 tests which include the motility test (Tittsler and Sandholzer, 1936), methyl red test (Aneja, 1996), sugar fermentation test (sucrose, lactose) (Atlas, 1993), nitrate reduction test (Dickey and Kelman, 1988), starch hydrolysis test (Aneja, 1996), catalase test (Dickey and Kelman, 1988), oxidase test (Dickey and Kelman, 1988), oxygen requirement (aerobic/anaerobic) (Prescott, 2002), citrate utilization test (Atlas, 1993), Voges-Proskauer-VP test (Cruickshank, 1963), Lysine decarboxylase test (Downes, 2001), triple sugar iron test and urea test (Woodland, 2004), respectively.

Results

The total of 8 bacterial strains such as MZ 1, MZ 2, MZ 3, MZ 4, MZ 5, MZ 6, MZ 7, MZ 8, MZ 9, MZ 10 and MZ 11 were isolated from the rhizospheric area of peanut soils. The results showed that the colonies morphology of those isolated strains (MZ 1-11) were small, moderate, punctiform large in sizes; circular, irregular, circular rhizoid, filamentous, entire in margins; cream, white, pale green and yellow in color; raised and flat in elevation and form; shiny and dull in pigments on agar, respectively. Those bacterial strains were short-rod, rod in their cell morphologies, facultative anaerobic, aerobic, anaerobic. The results of colony morphology, cell morphology and biochemical tests for the isolated bacterial strains were shown in Table 1-3 and Figure 1-21.

Table 1. Colony morphology of the isolated strains

Isolated Strains	Size of Colony	Margin	Color	Elevation and Form	Pigment on Agar
MZ 1	large	irregular	cream	flat	shiny
MZ 2	large	filamentous	cream	flat	dull
MZ 3	moderate	irregular	cream	raised	dull
MZ 4	small	circular	cream	raised	dull
MZ 5	large	rhizoid	white	flat	dull
MZ 6	large	filamentous	white	flat	shiny
MZ 7	punctiform	circular	yellow	raised	shiny
MZ 8	small	circular	yellow	raised	shiny
MZ 9	small	irregular	cream	flat	shiny
MZ 10	moderate	entire	Pale green	raised	shiny
MZ 11	small	entire	white	raised	shiny

Table 2. Cell morphology of the isolated strains

Isolated Strains	Gram Staining	Cell Morphology	Aerobic / Facultative/ Anaerobic
MZ 1	+	Short-rod	F. anaerobic
MZ 2	+	rod	F. anaerobic
MZ 3	+	rod	F. anaerobic
MZ 4	+	rod	Facultative
MZ 5	+	Short-rod	Facultative
MZ 6	+	Short-rod	Facultative
MZ 7	+	Short-rod	aerobic
MZ 8	+	rod	aerobic
MZ 9	+	rod	Facultative
MZ 10	+	rod	Facultative
MZ 11	+	rod	Facultative

Positive = +, negative = –, F. anaerobic = Facultative anaerobic

Table 3. Biochemical tests for the isolated strains

No.	Biochemical Tests	Isolated Strains										
		MZ 1	MZ 2	MZ 3	MZ 4	MZ 5	MZ 6	MZ 7	MZ 8	MZ 9	MZ 10	MZ 11
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 = +, negative = –

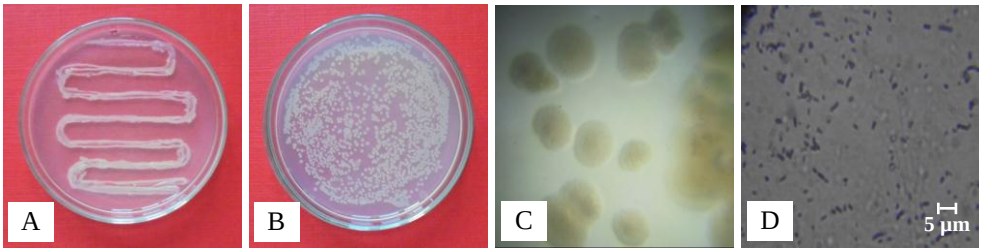

Figure 1. Cultural characteristics and cell morphology of MZ 1 on agar medium [A. Culture (Streaks Method), B. Colonies, C. Colony characters, D. Cell Morphology]

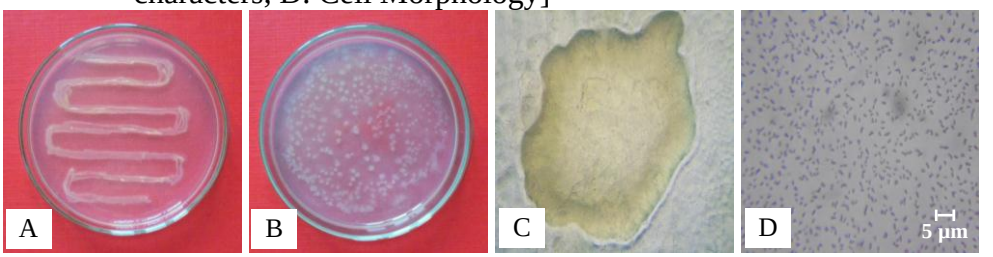

Figure 2. Cultural characteristics and cell morphology of MZ 2 on agar medium [A. Culture (Streaks Method), B. Colonies, C. Colony characters, D. Cell Morphology]

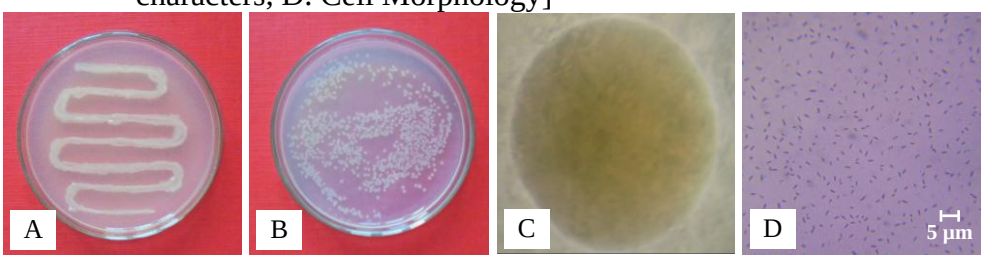

Figure 3. Cultural characteristics and cell morphology of MZ 3 on agar medium [A. Culture (Streaks Method), B. Colonies, C. Colony characters, D. Cell Morphology]

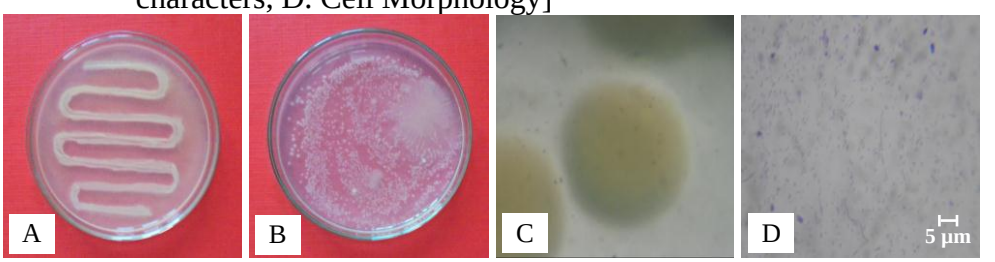

Figure 4. Cultural characteristics and cell morphology of MZ 4 on agar medium [A. Culture (Streaks Method), B. Colonies, C. Colony characters, D. Cell Morphology]

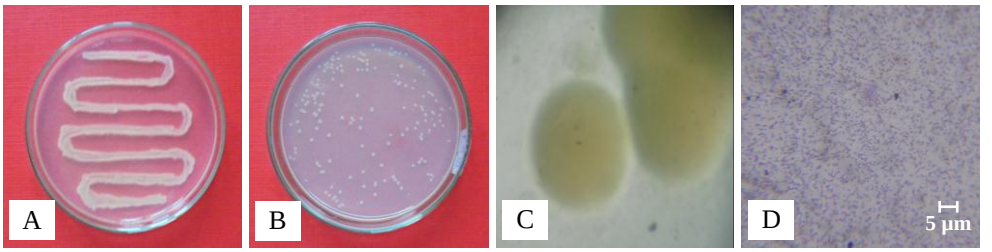

Figure 5. Cultural characteristics and cell morphology of MZ 5 on agar medium [A. Culture (Streaks Method), B. Colonies, C. Colony characters, D. Cell Morphology]

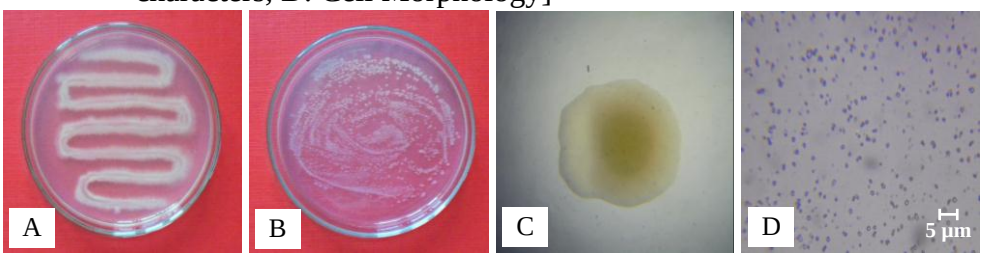

Figure 6. Cultural characteristics and cell morphology of MZ 6 on agar medium [A. Culture (Streaks Method), B. Colonies, C. Colony characters, D. Cell Morphology]

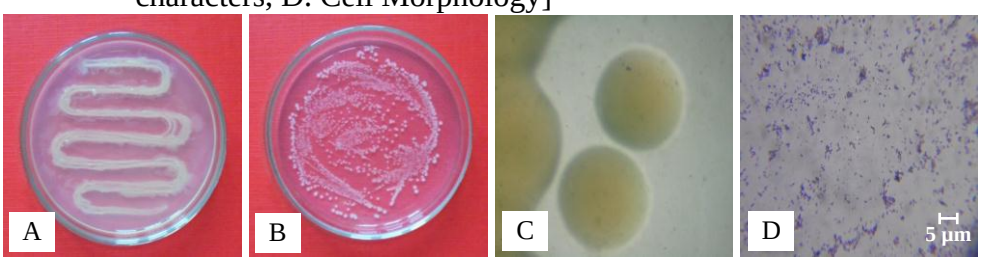

Figure 7. Cultural characteristics and cell morphology of MZ 7 on agar medium [A. Culture (Streaks Method), B. Colonies, C. Colony characters, D. Cell Morphology]

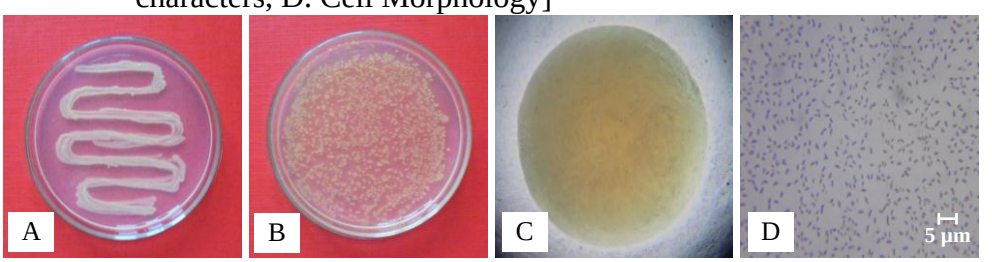

Figure 8. Cultural characteristics and cell morphology of MZ 8 on agar medium [A. Culture (Streaks Method), B. Colonies, C. Colony characters, D. Cell Morphology]

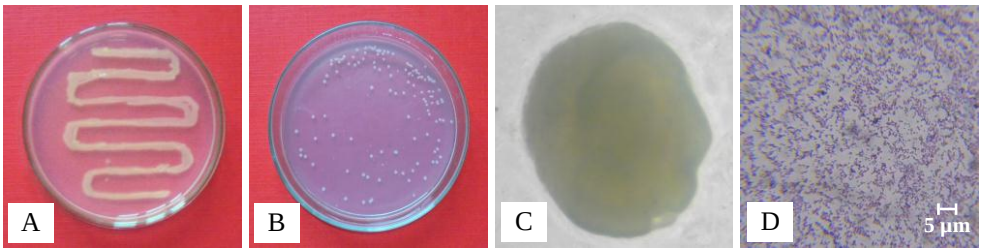

Figure 9. Cultural characteristics and cell morphology of MZ 9 on agar medium [A. Culture (Streaks Method), B. Colonies, C. Colony characters, D. Cell Morphology]

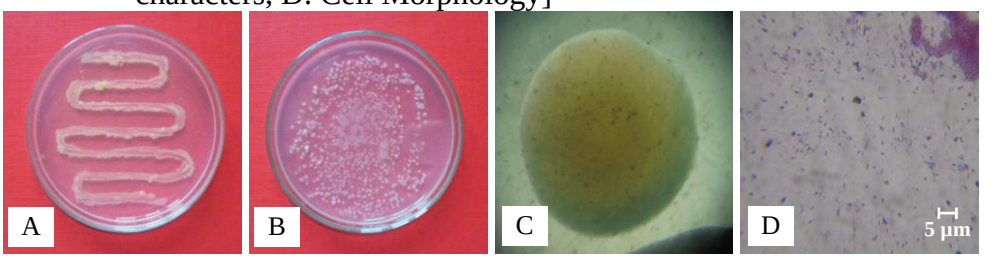

Figure 10. Cultural characteristics and cell morphology of MZ 10 on agar medium [A. Culture (Streaks Method), B. Colonies, C. Colony characters, D. Cell Morphology]

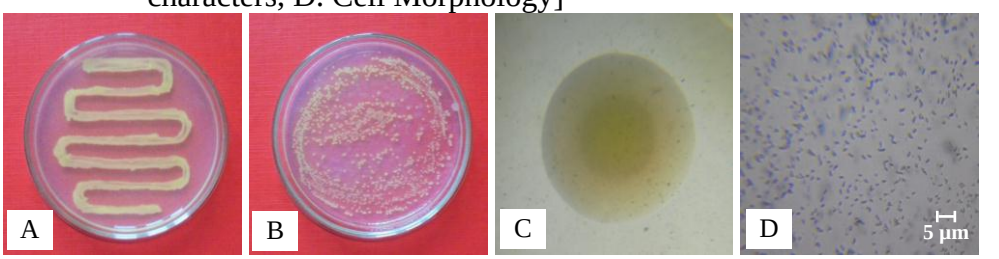

Figure 11. Cultural characteristics and cell morphology of MZ 11 on agar medium [A. Culture (Streaks Method), B. Colonies, C. Colony characters, D. Cell Morphology]

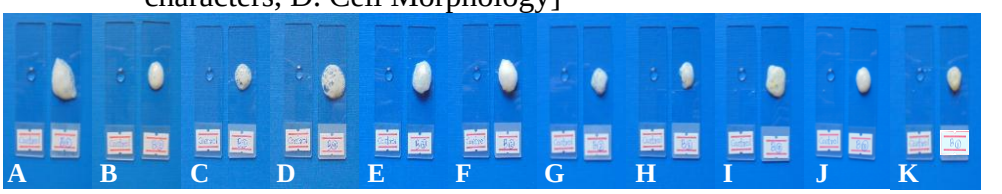

Figure 9. Biochemical test for Catalase test [A. MZ 1 (Positive), B. MZ 2 (Positive), C. MZ 3 (Positive), D. MZ 4 (Positive), E. MZ 5 (Positive), F. MZ 6 (Positive), G. MZ 7 (Positive), H. MZ 8 (Positive), I. MZ 9 (Positive), J. MZ 10 (Positive), K. MZ 11 (Positive)].

Figure 10. Biochemical test for 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 (Negative), G. MZ 7 (Positive), H. MZ 8 (Positive), I. MZ 9 (Positive), J. MZ 10 (Positive), K. MZ 11 (Negative)].

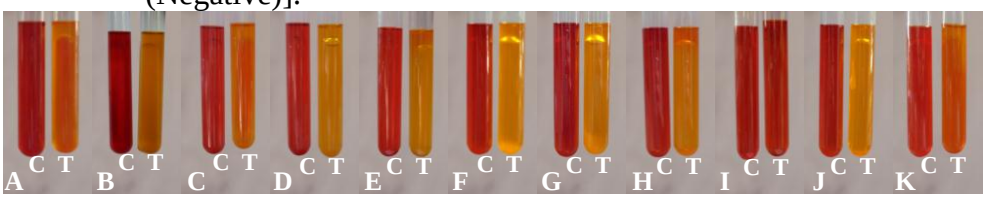

Figure 11. Biochemical test for sugar fermentation test (lactose) [A. MZ 1 (Positive), B. MZ 2 (Positive), C. MZ 3 (Positive), D. MZ 4 (Positive), E. MZ 5 (Positive), F. MZ 6 (Positive), G. MZ 7 (Positive), H. MZ 8 (Positive), I. MZ 9 (Negative), J. MZ 10 (Positive), K. MZ 11 (Positive)]. C = control, T = treatment

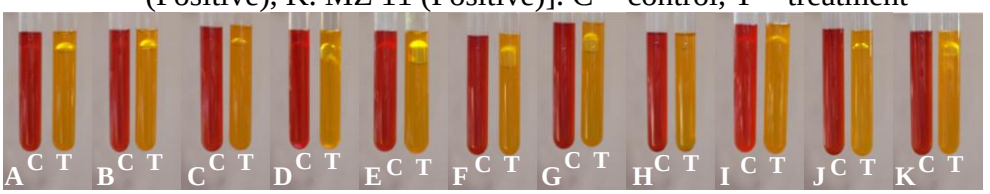

Figure 12. Biochemical test for sugar fermentation test (sucrose) [A. MZ 1 (Positive), B. MZ 2 (Positive), C. MZ 3 (Positive), D. MZ 4 (Positive), E. MZ 5 (Positive), F. MZ 6 (Positive), G. MZ 7 (Positive), H. MZ 8 (Positive), I. MZ 9 (Positive), J. MZ 10 (Positive), K. MZ 11 (Positive)]. C = control, T = treatment

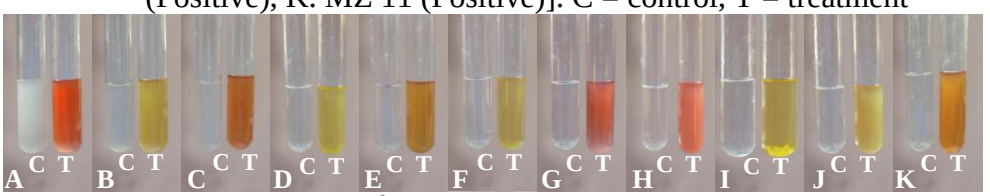

Figure 13. Biochemical test for methyl red (MR) test [A. MZ 1 (Positive), B. MZ 2 (Negative), C. MZ 3 (Positive), D. MZ 4 (Negative), E. MZ 5 (Negative), F. MZ 6 (Negative), G. MZ 7 (Positive), H. MZ 8 (Positive), I. MZ 9 (Negative), J. MZ 10 (Negative), K. MZ 11 (Positive)]. C = control, T = treatment.

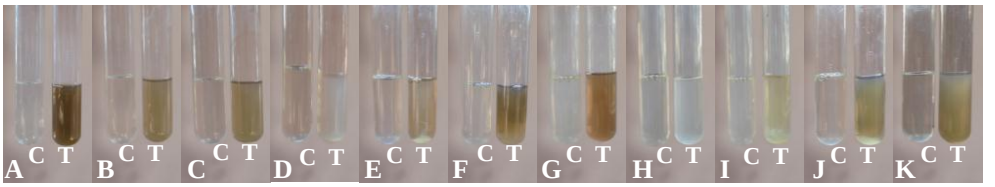

Figure 14. Biochemical test for Voges-Proskauer (VP) test [A. MZ 1 (Negative), B. MZ 2 (Negative), C. MZ 3 (Negative), D. MZ 4 (Negative), E. MZ 5 (Negative), F. MZ 6 (Negative), G. MZ 7 (Negative), H. MZ 8 (Negative), I. MZ 9 (Negative), J. MZ 10 (Negative), K. MZ 11 (Negative)]. C = control, T = treatment.

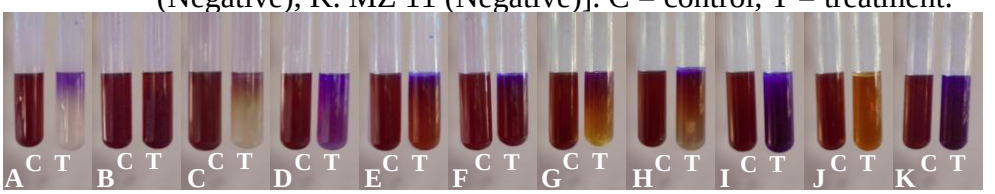

Figure 15. Biochemical test for Lysine test [A. MZ 1 (Positive), B. MZ 2 (Negative), C. MZ 3 (Positive), D. MZ 4 (Positive), E. MZ 5 (Positive), F. MZ 6 (Positive), G. MZ 7 (Positive), H. MZ 8 (Positive), I. MZ 9 (Positive), J. MZ 10 (Negative), K. MZ 11 (Positive)]. C = control, T = treatment.

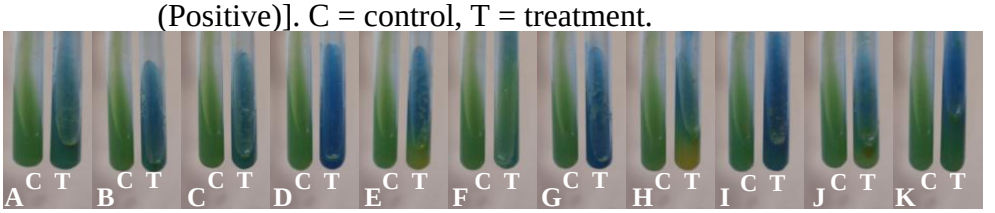

Figure 16. Biochemical test for Citrate test [A. MZ 1 (Positive), B. MZ 2 (Positive), C. MZ 3 (Positive), D. MZ 4 (Positive), E. MZ 5 (Positive), F. MZ 6 (Positive), G. MZ 7 (Positive), H. MZ 8 (Positive), I. MZ 9 (Positive), J. MZ 10 (Positive), K. MZ 11 (Positive)]. C = control, T = treatment.

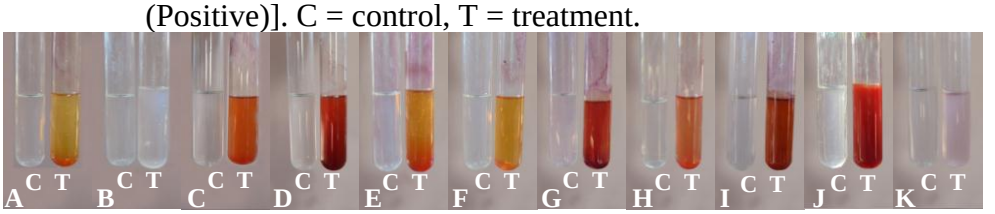

Figure 17. Biochemical test for Nitrate test [A. MZ 1 (Positive), B. MZ 2 (Negative), C. MZ 3 (Positive), D. MZ 4 (Positive), E. MZ 5 (Positive), F. MZ 6 (Positive), G. MZ 7 (Positive), H. MZ 8 (Positive), I. MZ 9 (Positive), J. MZ 10 (Positive), K. MZ 11 (Negative)]. C = control, T = treatment.

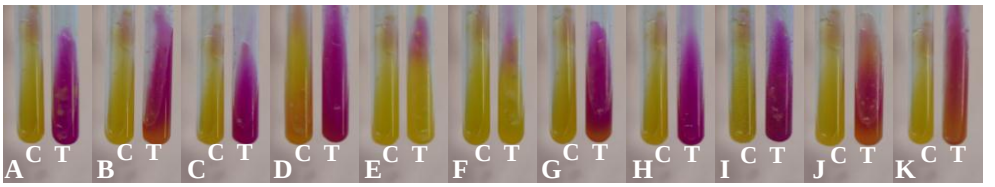

Figure 18. Biochemical test for Urease test [A. MZ 1 (Positive), B. MZ 2 (Positive), C. MZ 3 (Positive), D. MZ 4 (Positive), E. MZ 5 (Negative), F. MZ 6 (Negative), G. MZ 7 (Positive), H. MZ 8 (Positive), I. MZ 9 (Positive), J. MZ 10 (Positive), K. MZ 11 (Positive)]. C = control, T = treatment.

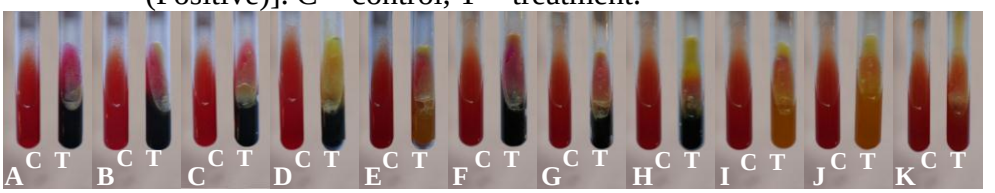

Figure 19. Biochemical test for triple sugar test [A. MZ 1 (Positive), B. MZ 2 (Positive), C. MZ 3 (Positive), D. MZ 4 (Positive), E. MZ 5 (Positive), F. MZ 6 (Positive), G. MZ 7 (Positive), H. MZ 8 (Positive), I. MZ 9 (Positive), J. MZ 10 (Positive), K. MZ 11 (Positive)]. C = control, T = treatment

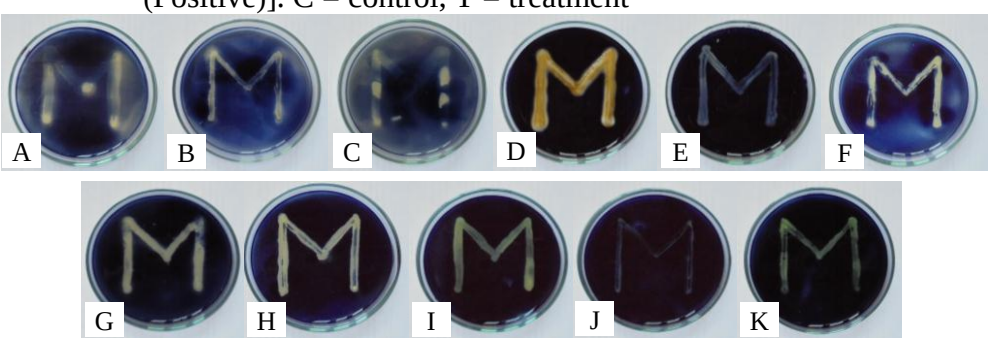

Figure 20. Biochemical test for starch hydrolysis test [A. MZ 1 (Positive), B. MZ 2 (Positive), C. MZ 3 (Positive), D. MZ 4 (Positive), E. MZ 5 (Positive), F. MZ 6 (Positive), G. MZ 7 (Positive), H. MZ 8 (Positive), I. MZ 9 (Positive), J. MZ 10 (Positive), K. MZ 11 (Negative)]

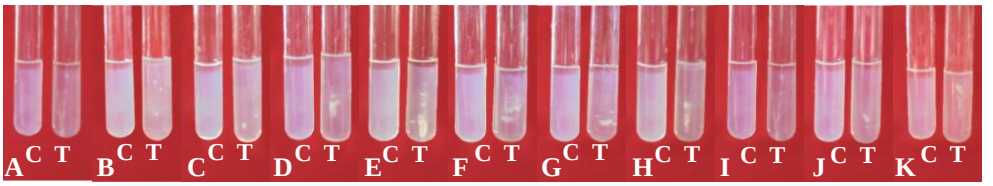

Figure 21. Motality tests of the isolated strains [A. MZ 1 (Positive), B. MZ 2 (Positive), C. MZ 3 (Positive), D. MZ 4 (Positive), E. MZ 5 (Positive), F. MZ 6 (Positive), G. MZ 7 (Positive), H. MZ 8 (Positive), I. MZ 9 (Positive), J. MZ 10 (Positive), K. MZ 11 (Positive)]. C = control, T = treatment.

Discussion and Conclusion

Most of the soil inhabitants are aerobic, the organisms are found in greater number in the surface layers. The numbers decrease as the depth of the soil increases. Microorganisms can be used to determine the bioavailability of a given chemical compounds in soil (Bating *et al*. 2008).

In this study, the rhizospheric soil samples were randomly collected from the peanut fields of Shangalay Kyun Village, Amarapura Township, Mandalay Region during August 2016. This study was carried out at Microbiology Laboratory of Botany Department, University of Mandalay from August 2016 to February 2017. After arrival to Microbiology Laboratory, the collected soil samples were immediately cultured on the ATCC 552 medium to isolate the bacterial strains.

The present findings showed that total of eight bacterial strains were isolated and identified from the rhizospheric soil of peanut fields. The isolated strains were designated as MZ 1-11.

To identify the genus level, all isolates were characterized by colony morphology, microscopical characters and biochemical tests. The colony of MZ 1 to 11 were large, moderate, small and punctiform respectively in the size. Their colony margins were irregular, filamentous, circular and rhizoid, and colony's color were cream, white and yellow. In the elevation and form, MZ 1-11 were flat and raised. MZ 1, 6 - 11 were shiny pigment and other strains (MZ 2 - 5) were dull on the agar medium. South Jakota Department of Health, Logan (2012) reported that colonies of *Bacillus* were flat or

slightly convex with irregular edges. These characters were agreed with in the present study.

The strains were gram-positive, rod and short rod, facultative anaerobic, motile, catalase positive, oxidase positive except MZ 6 and 11, acid was produced from sucrose and lactose test. All strains were negative in MR⁄VP test except MZ 1, 3, 7, 8 and 11; citrate positive; lysine positive and negative in MZ 2 and 10; urea positive; H_2S was produced in MZ 1 to 8; all stain hydrolyzed the starch. These characters were agreed with Waites *et al*. (2008) who described that *Bacillus* species are gram positive, endospore forming, rod-shaped bacteria which are usually motile; they are aerobic or facultative anaerobic and catalase positive.

These results were matched with the description of Bergey's Manual of Determinative Bacteriology of Buchanan & Gibbons (1974). According to the results, MZ 1-11were identified as genus *Bacillus*. Kuta (2008) also reported that members of the genus *Bacillus* are generally found in soil and represent a wide range of physiological abilities, allowing the organism to grow in every environment and compete desirably with other organisms within the environment due to its capability to form extremely resistant spores and produce metabolites that have antagonistic effects on other microorganisms.

Thus, it would be concluded that the present findings of those isolated bacterial strains (MZ 1-11) can be noted as the *Bacillus* bacterial strains. Those bacterial strains would be isolated from the rhizopheric soil of peanut fields and identified as *Bacillus* 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.

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