

ISOLATION AND CHARACTERIZATION OF SELECTED SOIL BACTERIUM SY-7 FROM MYINT THA TOWNSHIP, MANDALAY REGION

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Abstract

The present paper was focused on the isolation and characterization of selected soil bacterium. Soil samples were collected from three different area of Yoar Thit village of Myint Tha Township, Mandalay Region and cultured on FLO medium and Nutrient Agar Medium. The total of ten bacterial colonies were obtained and designated as SY-1 to SY-10. Antimicrobial activities of all strains were carried out by agar well diffusion assay on 5 test organisms. Among them, SY-7 showed the highest antifungal activity (32.26 mm) on *Candida albicans*. Therefore, SY-7 was selected and identified by morphological, microscopical and biochemical characteristics. In the morphological characterization, SY-7 was medium in size, rod-shaped, margin entire, spore present and cream colour. According to the results of biochemical characters, SY-7 was characterized as the genus *Bacillus*. This research can provide screening the antimicrobial activity and identification of soil bacteria by using the different biochemical characters.

Keywords: Soil Bacteria, Antimicrobial activity, Biochemical characterization

Introduction

Soil microorganisms, such as bacteria, and fungi, control ecosystem functioning through decomposition and nutrient cycling and may serve as indicators of land-use changes and ecosystem health (Balser *et al.*, 2010). Microbes are very small living organisms, so small that most of them are invisible (Subba, 1999).

Soil bacteria are source of higher members of bioactive natural products with biological activity which are extensively used as pharmaceutical and agrochemicals. Infectious diseases are a clear cut threat to the physical health and economic well-being of the world irrespective of site of residence (Young, 2007). Most of the Antibiotics in currents use for the treatment of various infections diseases are microbial products. Antibiotics resistance against infections diseases has increased in recent years (Tawish *et al.*, 2012).

Bacilli are rod-shaped, Gram-positive, speculating aerobes as facultative anaerobes. Most bacilli are saprophytes. Each bacterium creates only one spore, which is resistant to heat, cold radiation, desiccation and disinfectants.

The most commonly used biochemical tests involve the observation of whether as not a growth of the bacterium in liquid nutrient medium will ferment particular sugar such as glucose, lactose or mannitol.

Bacillus spp, produces many kinds of antibiotics which share a full range of such antimicrobial activities as bacterian, pamalin and Gramcidin (Todar, 2005).

In the present study, soil bacterium *Bacillus* was isolated from three different areas of Yoar Thit village of Myint Tha Township. However, nobody has carried out the antimicrobial activity and identification of soil bacteria from above places. Therefore, antimicrobial activity and identification of *Bacillus* sp was mainly studied in this research.

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Materials and Methods

Study area and collection of soil samples

Soil samples were collected from three different stations of Yoar Thit Village, Myint Tha Township, Mandalay Region. Soil samples were collected from 0-3 inches, 1-6 inches and under 6 inches deep from each of these stations using a sterile spatula. The experiments were carried out at the Microbiology Laboratory of Biotechnology and Development Center of Patheingyi University.

Isolation of Bacteria from the Soil Samples (Altas, 1993)

The soil Bacteria were isolated by serial dilution method and media such as FLO Medium and Nutrient Agar Medium.

Medium used for Isolation of Bacteria

FLO Medium (Altas, 1993)		Nutrient Agar Medium (Altas, 1993)	
Casein	10.0 g	Peptone	5.0 g
Peptone	10.0 g	NaCl	5.0 g
K ₂ HPO ₄	1.5 g	Yeast extract	2.0 g
MgSO ₄ . 7H ₂ O	1.5 g	Agar	15.0 g
Agar	15.0 g	Beef extract	1.0 g
Distilled Water	1000 mL	Distilled Water	1000 mL
pH	5.0	pH	5.0

Medium used for Antimicrobial Activity

Seed and Fermentation Medium Nutrient, Broth Medium (Dubey and Mahesh Wari 2007)		Assay Medium Glucose Yeast Peptone (GYP) Medium (Atlas, 1993)	
Peptone	5.0 g	Glucose	10 g
Beef extract	3.0 g	Yeast extract	3 g
Sodium chloride	5.0 g	Peptone	2 g
Yeast extract	10.0 g	Agar	16 g
Distilled water	1000 mL	Distilled water	1000 mL
pH	6.8-7.2	pH	6.5

After autoclaving, Nystatin (1.5 mL) was added to the medium.

Serial Dilution Method of Soil Samples (Collins, 1965)

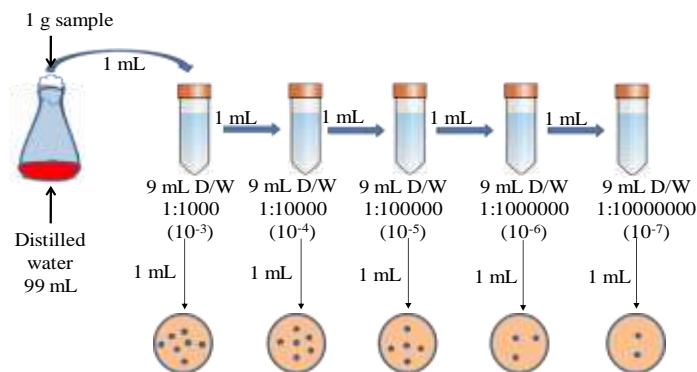


Figure 1 Serial dilution method for soil samples (Collins, 1965)

Screening of Antimicrobial Activity by Agar Well Method (Collins, 1965)

This method was used for the antimicrobial activity by seven test organisms. The assay medium (glucose – 1.0 g, yeast extract – 0.3 g, peptone – 0.2 g, agar – 1.6 g) was utilized for these bacteria. Isolated strains were subjected with antimicrobial activity by agar well method. Cork borer was used to make the wells (8 mm in diameter) in the autoclave basal antimicrobial test medium.

Well impregnated with 1-5 days old culture fermented broth (0.1 mL) were incubated at room temperature for 24-48 hours. After 24-48 hours of incubation, the clear zones were measured. Clear zone surrounding the test wells indicated the presence of antimicrobial activities which inhibit the growth of the test organisms selectively.

Table 1 Test organisms used in antimicrobial activities (NITE, 2004)

Sr No.	Test organisms	Infection
1.	<i>Agrobacterium tumefaciens</i> NITE 09678	Plant disease
2.	<i>Bacillus subtilis</i> IFO 90571	Fever
3.	<i>Bacillus pumilus</i> IFO 90571	Wound and burn infection
4.	<i>Candida albicans</i> NITE 09542	Candidosis
5.	<i>Escherichia coli</i> AHU 5436	Cholera, Diarrhea and vomiting, urinary tract infections

NITE = National Institute of Technology Evaluation

PRD = Pharmaceutical Research Development (Ministry of Industry)

Identification of Selected Bacterium

The identification of isolated bacterial strains were carried out by 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, maltose) (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), Urea test (Woodland, 2004), respectively.

Identification of selected bacterium SY-7

KB013-1KT, HiBacillus™ Identification Kit

Result interpretation chart						
No.	Test	Reagents to be added after incubation	Principle	Original colour of the medium	Positive reaction	Negative reaction
1	Methyl red	—	Methyl red utilization	Bluish green	Dark Blue	Bluish green
2	Voges-Proskauer's	1-2 drops of Barit reagent A and 1-2 drops of Barit reagent B	Detects acetoin production	Colourless/ Light yellow	Pinkish red	Colourless/ Light colour
3	Citrate	—	Citrate utilization	Light Green	Dark Blue	Light Green
4	ONPG	—	Detects Beta galactosidase	Colourless	Yellow	Colourless
5	Nitrate Reduction	1-2 drops of sulphuric acid and 1-2 drops of N, N-Dimethyl-1-Naphthylamine	Detects Nitrate reduction	Colourless / Light yellow	Pinkish red	Colourless
6	Catalase	3% H ₂ O ₂ solution	Detects Catalase activity	Colourless	Efferescence coming out from the foog	No Efferescence seen
7	Arginine	—	Arginine utilization	Dark Green to Light Purple	Purple / Dark Purple	No change in color or yellow
8	Sucrose	—	Carbohydrate utilization	Pinkish Red / Red	Yellow	Red / Pink
9	Mannitol	—	Carbohydrate utilization	Pinkish Red / Red	Yellow	Red / Pink
10	Starch	—	Carbohydrate utilization	Pinkish Red / Red	Yellow	Red / Pink
11	Arabinose	—	Carbohydrate utilization	Pinkish Red / Red	Yellow	Red / Pink
12	Trehalose	—	Carbohydrate utilization	Pinkish Red / Red	Yellow	Red / Pink

Results

The total of 10 bacterial strains (SY-1 to 10) were isolated from the Yoar Thit Village, Myint Thar Township.

Table 2 Isolated Bacteria from Soil Samples

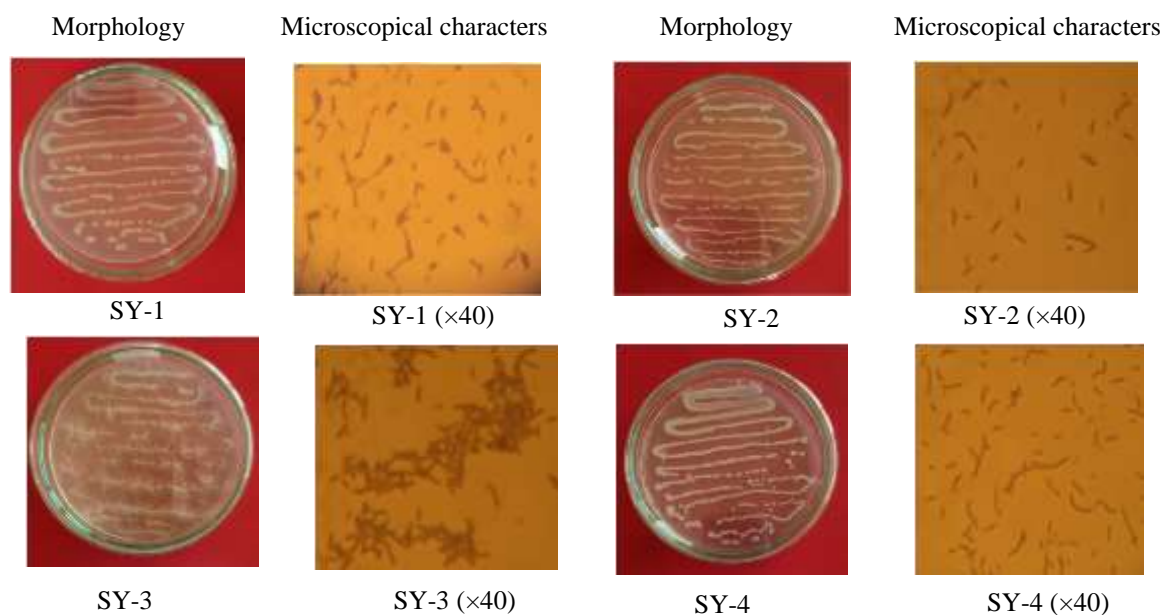
Soil Sample No.	FLO Medium	Nutrient Agar Medium
S - 1	SY 1 – 2 = 2	SY 3-4 = 2
S - 2	SY 5 – 6 = 2	SY 7 = 1
S - 3	SY 8 = 1	SY 9-10 = 2

Table 3 Colony and Cell Morphology of Isolated Bacteria

Isolated Bacteria	Shape	Size of Colony	Margin	Color	Elevation and Form	Cell Mor-phology	Gram Staining
SY - 1	Circular	Medium	Lobate	White	Flat	Rod	-
SY - 2	Circular	Medium	Entire	White	Flat	Rod	-
SY - 3	Filamentous	Medium	Rhizoid	White	Raise	Rod	-
SY - 4	Circular	Small	Entire	Cream	Flat	Rod	-
SY - 5	Circular	Small	Entire	Cream	Flat	Rod	-
SY – 6	Irregular	Large	Entire	Cream	Flat	Rod	-
SY – 7	Circular	Medium	Entire	Cream	Flat	Rod	+
SY – 8	Circular	Medium	Undulate	Cream	Flat	Rod	-
SY – 9	Circular	Large	Entire	Cream	Flat	Rod	-
SY - 10	Circular	Small	Entire	Cream	Flat	Rod	+

Small < 2mm diameter/ Medium between 2mm and 5mm diameter

Large > 5mm diameter + = Gram positive - = Gram negative



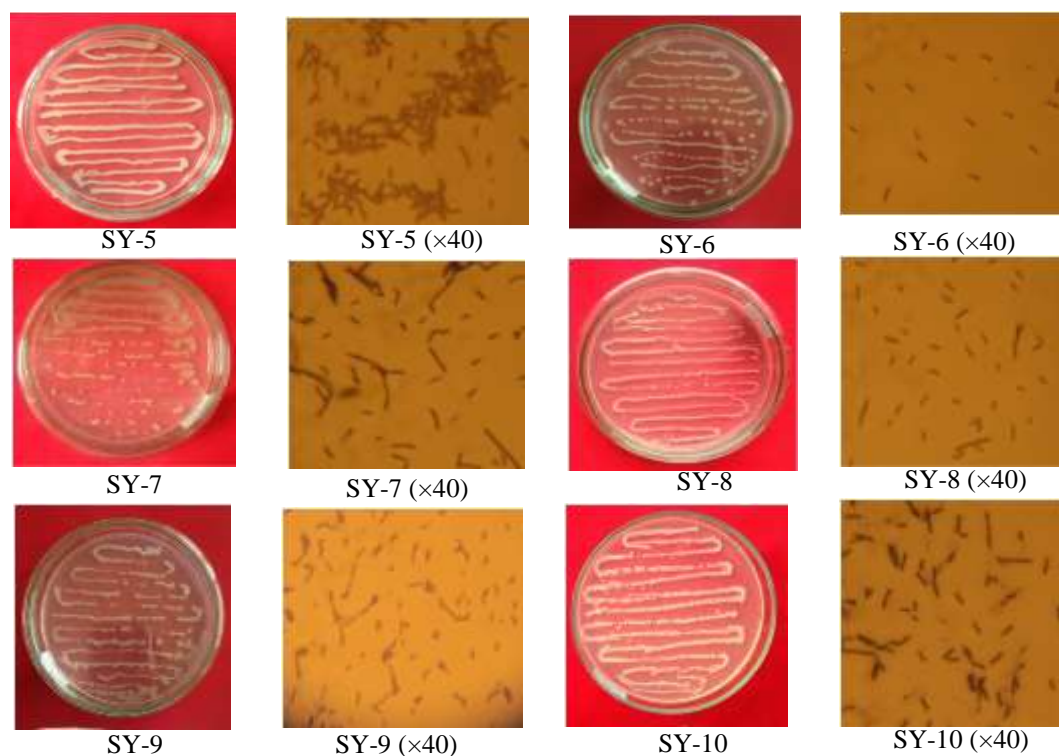


Figure 2 Cultural Character and Cell morphology of Isolated Bacteria SY-1 to SY-10

Isolated bacteria and their Antimicrobial Activity

Seven isolates (SY-1, SY-2, SY-4, SY-6, SY-7, SY-8 and SY-9) had antimicrobial activity and SY-7 showed the highest antifungal activity (32.26) against on *Candida albicans* followed by 30.44 mm on *Bacillus subtilis*. Other bacteria (SY-3, 5 and 10) could not produce antimicrobial metabolites.

Table 4 Antimicrobial Activity of Isolated Bacteria

No.	Isolated bacteria	Test Organisms and Antimicrobial Activity (mm)				
		<i>Agrobacterium tumefaciens</i>	<i>Bacillus pumilus</i>	<i>Bacillus subtilis</i>	<i>Candida albicans</i>	<i>E. coli</i>
1	SY - 1	21.41	23.31	15.61	15.76	16.99
2	SY - 2	19.79	19.89	21.22	23.66	19.46
3	SY - 3	-	-	-	-	-
4	SY - 4	18.77	19.17	18.22	21.86	18.82
5	SY - 5	-	-	-	-	-
6	SY - 6	16.11	17.86	19.21	14.03	16.33
7	SY-7	26.20	29.91	30.44	32.26	20.88
8	SY-8	14.21	16.33	17.18	16.77	14.11
9	SY-9	17.62	17.64	18.11	26.88	16.01
10	SY-10	-	-	-	-	-

The antimicrobial activity of these strains were tested by using five different test organisms. Seven strains showed the activity on *A. tumefaciens*, *B. pumilus*, *B. subtilis*, *C. albicans* and *E. coli*.

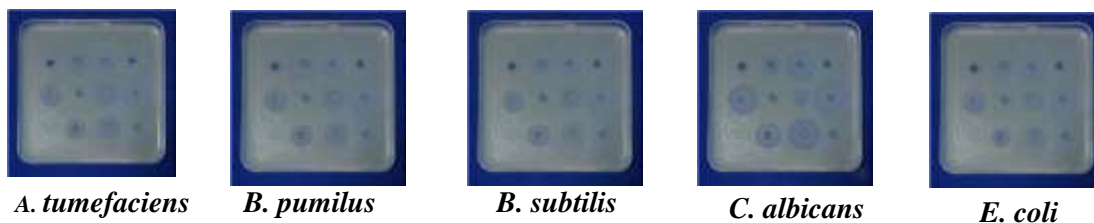


Figure 3 Antimicrobial Activity of Ten Isolated Bacteria

Therefore, SY-7 was selected and identified by colony morphology, Gram-staining and Biochemical characteristics. In the colony and cell morphology, SY-7 was medium in size, entire margin, cream colour, rod shaped and spore present.

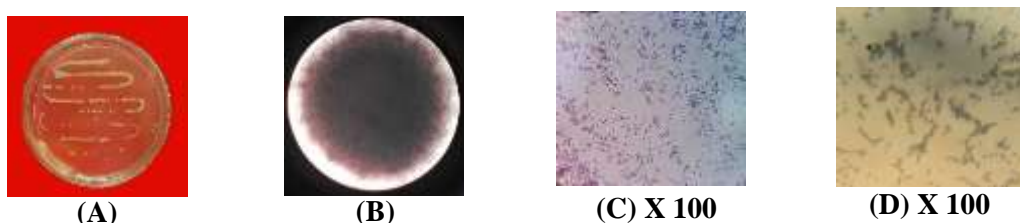


Figure 4 (A) Colony character (B) Colony morphology (C) Gram staining (D) Spore staining of selected bacterium SY-7

Table 5 Carbohydrate Fermentation of Selected Strain SY-7

Sugar sources	Responces Yellow colour	Acid production	Gas production
Sucrose	Change in medium	+	-
Glucose	Change in medium	+	-
Maltose	Change in medium	+	-
Xylose	Change in medium	+	-
Fructose	Change in medium	+	-

+ = acid and gas was produced - = acid and gas was not produced

The positive results of SY-7 were able to ferment the glucose, sucrose, maltose, xylose and fructose that is responsible for sugar fermentation and produced acid.

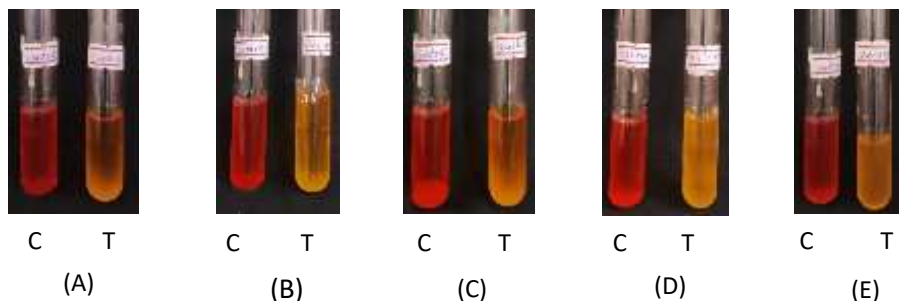


Figure 5 Carbohydrate Fermentation Test of Selected Bacterium (SY-7) A. Sucrose, B. Glucose, C. Maltose, D. Xylose, E. Fructose (All positive)

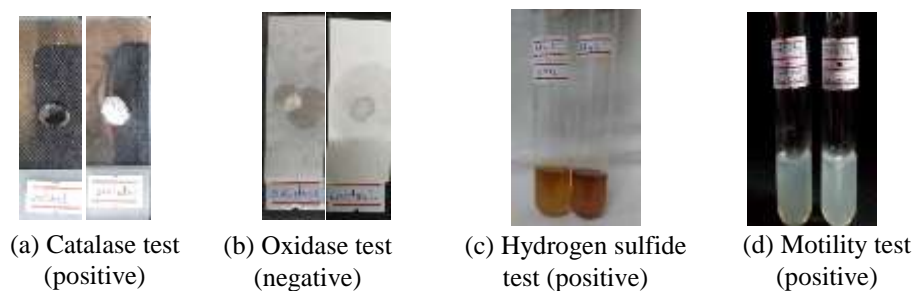
Table 6 Biochemical Characteristics of Selected Strain SY-7

No	Biochemical tests	Responses	Results
1	Urea hydrolysis test	No change in colours	-
2	Nitrate reduction test	Change in orange colour	+
3	Methyl red test	No change colour	-
4	Voges proskaucer test	Change in orange colour	+
5	Citrate utilization test	Medium change from green to blue	+
6	H ₂ S production test	Change black	+
7	Catalase test	Release free oxygen gas bubble	+
8	Oxidase test	No change color	-
9	Gelatin hydrolysis	No clear zone around the colony	-
10	Potato plug	Growth in streak line	+
11	starch hydrolysis		
	(i) Soluble starch	Clear zone around in streak line	+
	(ii) Tapioca powder	Clear zone around in streak line	+
	(iii) Sticky rice powder	Clear zone around in streak line	+
	(iv) Wheat powder	Clear zone around in streak line	+
	(v) Rice	Clear zone around in streak line	+
12	Caesin hydrolysis	Clear zone around the colony	+
13	Esterase activity	No colour change in medium	-
14	Salt tolerance test		
	(i) 1% NaCl	High growth	+
	(ii) 2% NaCl	High growth	+
	(iii) 3% NaCl	Moduate growth	+
	(iv) 4% NaCl	Moduate growth	+
	(v) 5% NaCl	Poor growth	-
	(vi) 6% NaCl	Poor growth	-
15	Triple Sugar Iron	Change in pink colour	+
16	Motility	Motile	+
17	Aerobic/Anaerobic Test	Facultative anaerobic	
18	PPA (Phenylalanine)	no change in colour	-

+ = Gram positive

- = Gram negative

In the biochemical characteristics properties, SY-7 was found to be positive in catalase reaction, motility, aerobic and anaerobic and hydrogen sulfide production. Oxidase test was negative.

**Figure 6** Biochemical Characteristics of Selected Bacterium (SY-7)

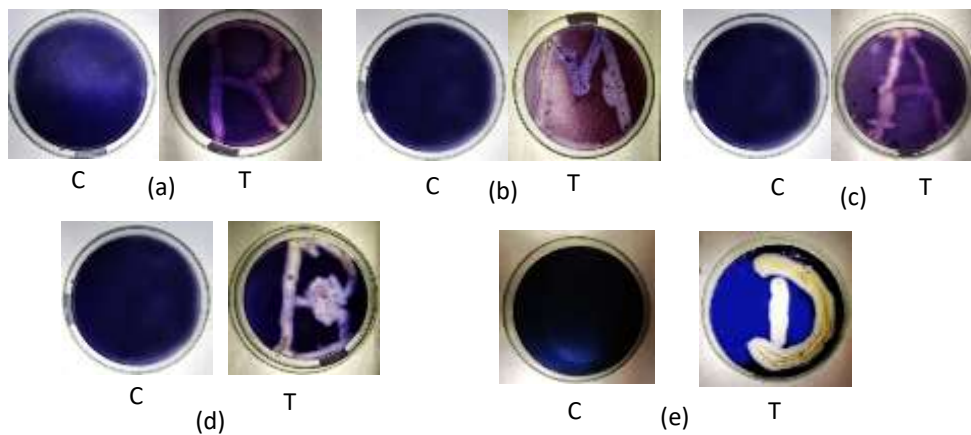


Figure 7 Starch Hydrolysis Test of Selected Bacterium SY-7 (a) Rice (positive), (b) Sticky rice (positive), (c) Wheat (positive), (d) Starch (positive), (e) Tapioca powder (positive)

SY-7 can grow well in 1% and 4% NaCl at room temperature. SY-7 was found to be positive in voges-proskauer (VP) and citrate utilization and negative in methyl red.

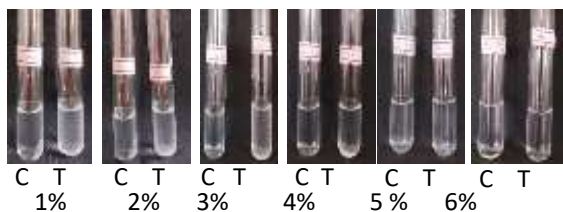


Figure 8 NaCl Tolerance Test of Selected Bacterium SY-7 (1%, 2% highest growth), (3%, 4% moderate growth), (5%, 6% poor growth)

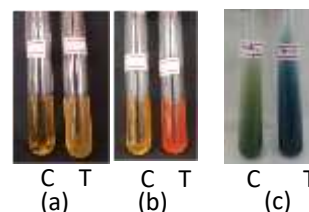


Figure 9. Biochemical Characteristics of Selected Bacterium SY-7 (a) Methyl red (negative), (b) Voges Proskauer (positive), (c) Citrate utilization (positive)

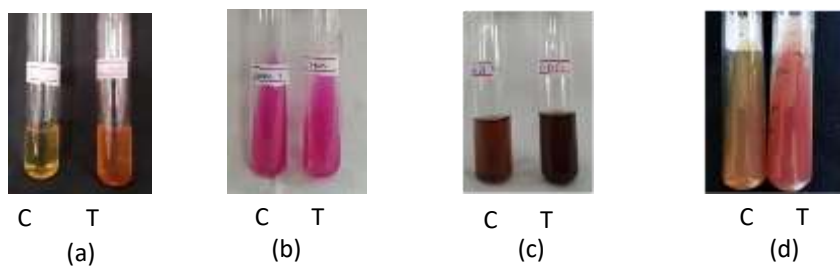


Figure 10 Biochemical Characteristics of Selected Bacterium SY-7 (a) Nitrate reduction (positive), (b) Urea hydrolysis (negative), (c) Phenylalanine (PPA) (negative), (d) Triple Sugar Iron (TSI) (positive)

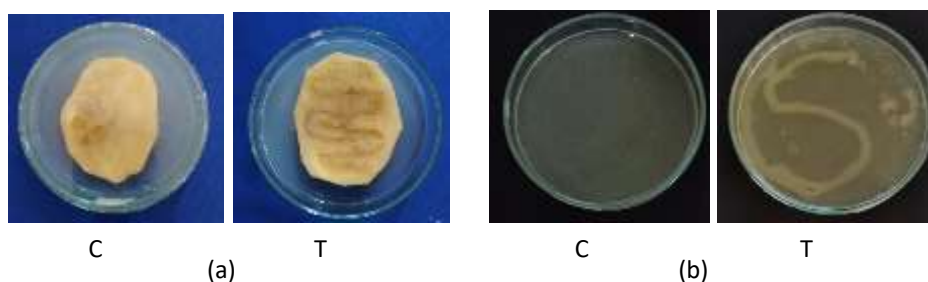


Figure 11 Potato Plug Test and Esterase Activity of Selected Bacterium SY-7 (a) Potato plug test (positive) (b) Esterase activity (negative)

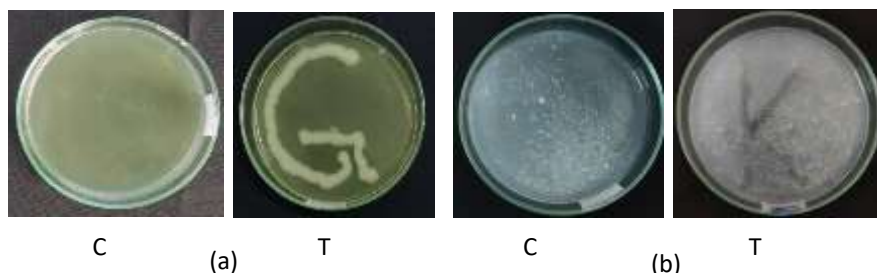


Figure 12 Gelatin (negative) and Casein hydrolysis (positive) Test of Selected Bacterium SY-7

Table 7 Identification of selected bacterium SY-7 (KB013-1KT, HiBacillus™ identification Kit)

No.	Test	Original Color of the medium	Result
1.	Malonate	Bluish green	Dark blue (+)
2.	Voges Proskauer's	Colourless/Light yellow	Pinkish red (+)
3.	Citrate	Light Green	Dark blue (+)
4.	ONPG	Colourless	Yellow (+)
5.	Nitrate Reduction	Colourless/Light yellow	Pinkish red (+)
6.	Catalase	Colourless	Effervescence coming out from the loop (+)
7.	Arginine	Olive Green to Light Purple	Purple/Dark purple (-)
8.	Sucrose	Pinkish Red/Red	Yellow (+)
9.	Mannitol	Pinkish Red/Red	Yellow (+)
10.	Glucose	Pinkish Red/Red	Yellow (+)
11.	Arabinose	Pinkish Red/Red	Yellow (+)
12.	Trehalose	Pinkish Red/Red	Yellow (+)

SY-7 was identified by KB013-1KT, HiBacillus™ Identification Kit and the results were the same with those of manual biochemical tests.

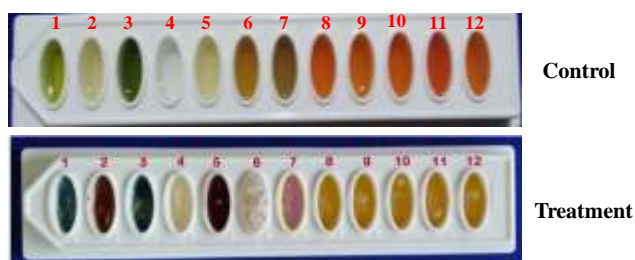


Figure 13 Identification of selected bacterium SY-7 (KB013-1KT, HiBacillus™ Identification Kit)

Discussion and Conclusion

Soil is a primary source of microorganisms. Soil bacteria and fungi have played a significant and an important role in antibiotic discovery. (Mashoria *et al.*, 2014)

In the course of the isolation of bacteria, three different samples were collected from Myit Tha Township, Mandalay region and 10 bacterial strains were obtained from these soil samples.

Two different media were employed in the investigation of the isolation of bacteria. Five strains were obtained from FLO medium and other five strains from Nutrient Agar medium.

Cell morphology of isolated strains was studied by Gram staining, colony characters and shape of cell. Among them, all strains were rod.

Hoorman, 2011 described that most of the bacteria belong to three main shapes: rod (rod shaped bacteria are called bacilli), sphere (sphere shaped bacteria are called cocci), and spiral (spiral shaped bacteria are called spirilla) and slender branching filaments called actinomycetes.

Antimicrobial activity of isolated bacterial strains were tested by agar well diffusion method on five test organisms and these strains showed different level of antimicrobial activities.

Among them, SY-7 exhibited the highest antimicrobial activity (32.26 mm) on *Candida albicans* followed by (30.44 mm) on *Bacillus subtilis* and (29.91 mm) on *Bacillus pumilus* respectively.

Shapiro, 2002 described that research on antimicrobial compounds as a new class of drugs has increased in the recent past as they exhibit both narrow and broad spectrum inhibitory activities against Gram-positive and Gram-negative bacteria.

In the characterization of SY-7, the results of colony character and biochemical characterization were similar to the previous research of Buchanan, 1974 and Vargar *et al.*, 2004.

Vargar *et al.*, 2004 reported that *Bacilli* are described as aerobic or facultative anaerobic, gram positive, rod-shaped, flagellated motile bacteria, catalase positive belong to the division Firmicutes with a wide ecological diversity mostly saprophytic they are commonly found in soil, dust, milk, plant surface, a few are animal or insect parasites or pathogen.

Moreover, SY-7 was identified by KB013-1KT, HiBacillus™ Identification Kit and the results were the same with those of manual biochemical tests.

Therefore, the selected bacterium SY-7 was characterized as the genus *Bacillus* spp.

Further study will be studied the purification and identification of isolated compounds and minimum inhibitory concentration (MIC).

Acknowledgements

Firstly, I wish to express our gratitude to Professor Dr Aye Aye Than, Head of Botany Department, Kyaukse University and Professor Dr Tin Tin Thein, Department of Botany, Kyaukse University for providing me an opportunity to do this work. Many thanks are due to my supervisor Dr Zar ZarYin, Associate Professor, Department of Botany, Bago University, for her valuable instructions, encouragement and overall supervisor for the successful completion of this research paper.

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