Preliminary Studies on Antimicrobial Activities of Actinomycetes from the Soils in Taunggyi Area, Myanmar

Win, O.Y.[1], Han Su Kyi[2], Hay Man Khin[3]

[1]*Botany Department, Panglong University, Southern Shan State, Myanmar*
[2]*Botany Department, Pakokku University, Magway Region, Myanmar*
[3]*Botany Department, Meiktila University, Mandalay Region, Myanmar*

***Abstract*— The present study involves the preliminary screening of actinomycetes for antimicrobial activities. Two soil samples were collected from Taunggyi area. They were serially diluted and the isolation was conducted by the selective medium, Glucose-yeast extract-beef-peptone (GYB) agar medium. A total of 10 actinomycetes (TG 1 to TG 10) with distinct characteristics were isolated from the soil samples. The preliminary screening for antimicrobial activity of actinomycete isolates was performed. The inhibitory effect of the isolated microorganism towards the growth of test microorganisms was observed. By antimicrobial analysis, it was found that isolate no. TG 4 showed the highest antimicrobial activity against Staphylococcus aureus, Bacillus pumilus and Candida albicans and isolate no. TG 7 and TG 8 exhibited the potent antifungal activity against Candida albicans after 3- day fermentation. Isolate no. TG 4 was found to have a broad spectrum of activity against all the test microorganisms except Bacillus subtilis and isolate no. TG 7 showed the high antibacterial activity against E. coli after 4-day fermentation. It was shown that isolate no. TG 7 and TG 8 had the highest antifungal activity against Candida albicans after 5-day fermentation. The present study indicated that soil actinomycetes had the active antimicrobial activity against six test microorganisms. It can be concluded that the confirmation of isolated strains as genus and species levels was going to be conducted.**

***Keywords*— preliminary screening, serial dilution, soil actinomycetes, antimicrobial activity, GYB agar medium.**

I. INTRODUCTION

Many scientists all over the world have screened, isolated and identified the different types of microorganisms and then applied in the production of food, beverages, enzymes, vitamins, organic acids and many pharmaceuticals. Myanmar is a developing country and rich in various types of resources including flora and fauna. There may be a high possibility of new types of microbes existing in the ecosystem of Myanmar. It is very important to find out a new method which can assure the most efficient and effective utilization of microbial resource and their production. During the year 1900 – 1919, Die, K.A., Conn, H.J., and Waksman, S.A., were interested in the predominantly occurrence of actinomycetes in soils and in other natural environments as in [2], [3], [12]. The introduction of synthetic media could broaden greatly their knowledge of the nature and occurrence of the actinomycetes. In 1914, Die, K.A., emphasized the growth characteristics of actinomycetes on synthetic media. The nature of the aerial mycelium, pigmentation of the colony, and the formation of soluble pigment were all controlled by the composition of the medium. In 1915, Waksman, S.A., treated the use of synthetic media for characterizing actinomycetes. Actinomycete colonies can easily be distinguished on the plate from those of fungi and true bacteria. They are compact, often leathery, giving a conical appearance, and have a dry surface. They are often covered with aerial mycelium [11], [12].

Among microorganisms, actinomycetes are the most important because of their capacity to produce numerous bioactive molecules including antibiotics and enzymes. They are prokaryotes having high G+C content in their deoxyribonucleic acid, with extremely various metabolic possibilities. The actinomycete family has extremely large genome, which has hundreds of transcription factors that control gene expression, so they have the large metabolic diversity. They are unique antibiotic producers, making three quarters of all known products; the *Streptomyces* are especially prolific and can produce a lot of antibiotics and other biologically active metabolites. Actinomycete population forms an important component of the soil microflora [9]. Actinomycetes can synthesize many different biologically active metabolites such as antibiotics, herbicides, pesticides and enzymes [6].

In the present research work, the geographical area of Taunggyi is of significant interest. Thus, the aim of the present study deals with screening of industrially important secondary metabolites, antibiotics.

II. MATERIALS AND METHODS

A total of 2 soil samples were collected from Taunggyi area in 2015 as listed in Table 1. Soil samples were collected in sterile plastic bags and brought to the laboratory. They are dried at room temperature for 7 days and stored in the sterile air-tight containers.

The isolation of actinomycetes was conducted by the following procedures:

1. One gram of soil samples was homogenized with 9 ml of distilled water. This is called 10^{-1} dilution (initial dilution).
2. One mililitre of this initial dilution was transferred into test tube containing 9 ml of distilled water to get 10^{-2} dilution.
3. One mililitre of 10^{-2} dilution was then transferred into further test tube containing 9 ml of distilled water to get 10^{-3} dilution.
4. This step was processed until 10^{-7} dilution prior to inoculation into the isolation plates.
5. Samples from the dilution series were cultured on GYB agar medium (also known as Emerson agar medium) (Waksman, S.A., 1961) and were incubated at 28°C for 1 – 2 weeks. The actinomycete colonies that appeared on petri plates were counted from $5^{th.}$-day onwards up to $14^{th.}$-day.
6. The colonies were re-streaked to get pure culture.
7. The pure isolates were cultured on agar slant in the test tubes.

A. Preparation of plates for antimicrobial activity of actinomycetes

The antimicrobial activities were performed by agar-well diffusion method. Nutrient agar was prepared according to the method described by Harley, J.P. & Prescott, L.M. (2002).

Nutrient agar was boiled and 20 – 25 ml of the medium was poured into a test tube. They were plugged with cotton wool and autoclaved at 121°C for 15 minutes. Then they were cool down to 60°C and poured into sterilized petridishes. Then 0.1 ml of spore suspension was added into the dishes and the agar was allowed to set for 30 minutes. Then 10 mm plate agar well was made with the sterilized cork borner. After that 0.2 ml of fermentation sample was introduced into the agar well and incubated at 28 dC for 24 hrs. The inhibition zone appeared around the agar-well indicated that the presence of antimicrobial activity. Six test microorganisms namely *Bacillus subtilis* (N.C.T.C. – 8236), *Staphylococcus aureus* (N.C.P.C. – 6371), *Pseudomonas aeruginosa* (6749), *Bacillus pumilus* (N.C.I.B. – 8982), *Candida albicans* and *Escherichia coli* (N.C.I.B. – 8134) were used at the Central Research and Development Center (CRDC) for the determination of antimicrobial activity.

III. RESULTS AND DISCUSSION

In the present study, ten different actinomycetes were gained from the soils in Taunggyi (Fig. 1).

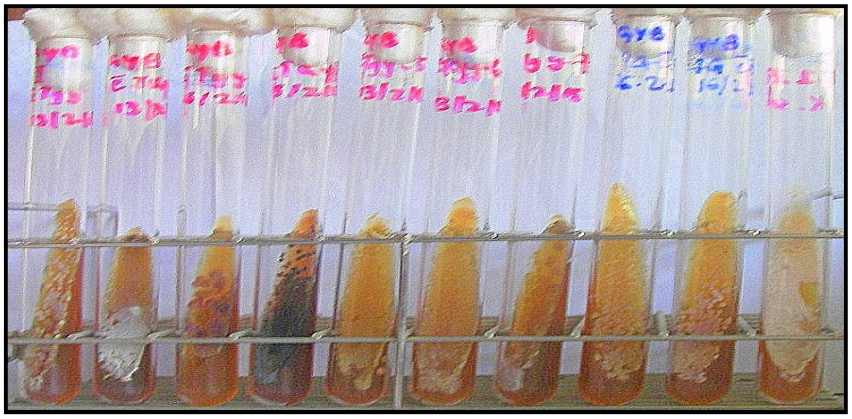
Actinomycete isolates TG 1 to TG 8 were isolated from soil sample 1 and the isolates TG 9 to TG 10 were from soil sample 2 as shown in Table 1. The colors of isolated actinomycete colonies were recorded as in [11], [12], as shown in Table 2.

Table 1:- SAMPLE COLLECTION SITES FOR THE ISOLATION OF ACTINOMYCETES

Soil Samples	Collection Sites	Actinomycete Isolates
1	N 20º 46' 16", E 97º 2' 30"	TG 1 – TG 8
2	N 20º 46' 8", E 97º 2' 30"	TG 9 – TG 10

Table 2:- AERIAL MASS COLOUR OF ISOLATED ACTINOMYCETE STRAINS

Isolates No.	Colony Color	Isolates No.	Colony Color
TG 1	Greyish White	**TG 6**	Whitish
TG 2	Dark Grey	**TG 7**	Grey
TG 3	Reddish Purple	**TG 8**	Yellowish Grey
TG 4	Black	**TG 9**	Light Grey
TG 5	Yellow	**TG 10**	Greyish Cream



TG 1 / TG 2 / TG 3 / TG4 / TG 5 / TG 6 / TG 7 / TG 8 / TG 9 / TG 10
Fig. 1:- Isolated actinomycete strains on agar slant in test tubes (Isolates no. TG 1 to TG 10)

A. Antimicrobial Activity of Actinomycete Isolates

The isolated acinomycetes were screened for antimicrobial activity after 3 day-fermentation (Table 3), after 4 day-fermentation (Table 4), and after 5 day-fermentation (Table 5). Among them, actinomycete isolate no. TG 4 showed the highest antimicrobial activity of 3-day fermentation against *Staphylococcus aureus*, *Bacillus pumilus* and *Candida albicans* (Plate 1:- Fig. i, ii, iii). Isolate no. TG 4 also showed the potent antimicrobial activity of 4-day fermentation against all test microorganisms except *Bacillus subtilis* (Plate 1:- Fig iv, v, vi; Plate 2:- Fig vii, viii). Isolate no. TG 7 and TG 8 showed high antibacterial activity against *E. coli* after 4-day fermentation and the potent antifungal activity against *Candida albicans* after 3-day and 5-day fermentation respectively (Plate 2:- Fig. ix, x, xi).

Atlas, R.M. (2010) stated that Emerson agar medium was used for the isolation, cultivation, and maintenance of members of the Actinomycetaceae, Streptomycetaceae and molds.

In this research work, 10 actinomycetes with distinct characteristics were isolated from the soils in Taunggyi. The findings in this study were in accordance with those studied by [2], [3], [11], [12], during the year 1900 – 1919 and those described by [1], [11], [12]. These actinomycete isolates were screened for their antimicrobial activity. After 3-day fermentation, isolate no. TG 4 exhibited the potent antimicrobial activity against *Staphylococcus aureus*, *Bacillus pumilus* and *Candida albicans* (Table 3; Plate 1:- Fig. i, ii & iii). After 4-day fermentation, isolate no. TG 4 showed the broad spectrum of antimicrobial activity against the six test microorganisms except *Bacillus subtilis* (Table 4; Plate 1:- Fig. iv, v, vi, Plate 2:- Fig. vii, & viii). After 3-day and 5-day fermentation, isolates no. TG 7 and TG 8 showed the highest antifungal activity against *Candida albicans* (Table 3 & 5; Plate 2:- Fig. ix & xi). After 4-fermentation, isolate no. TG 7 showed the high antibacterial activity against *E. coli* (Table 4; Plate 2:- Fig. x). The antimicrobial activities of the most active actinomycete isolates: TG 4, TG 7 & TG 8 were shown in Fig. 2.

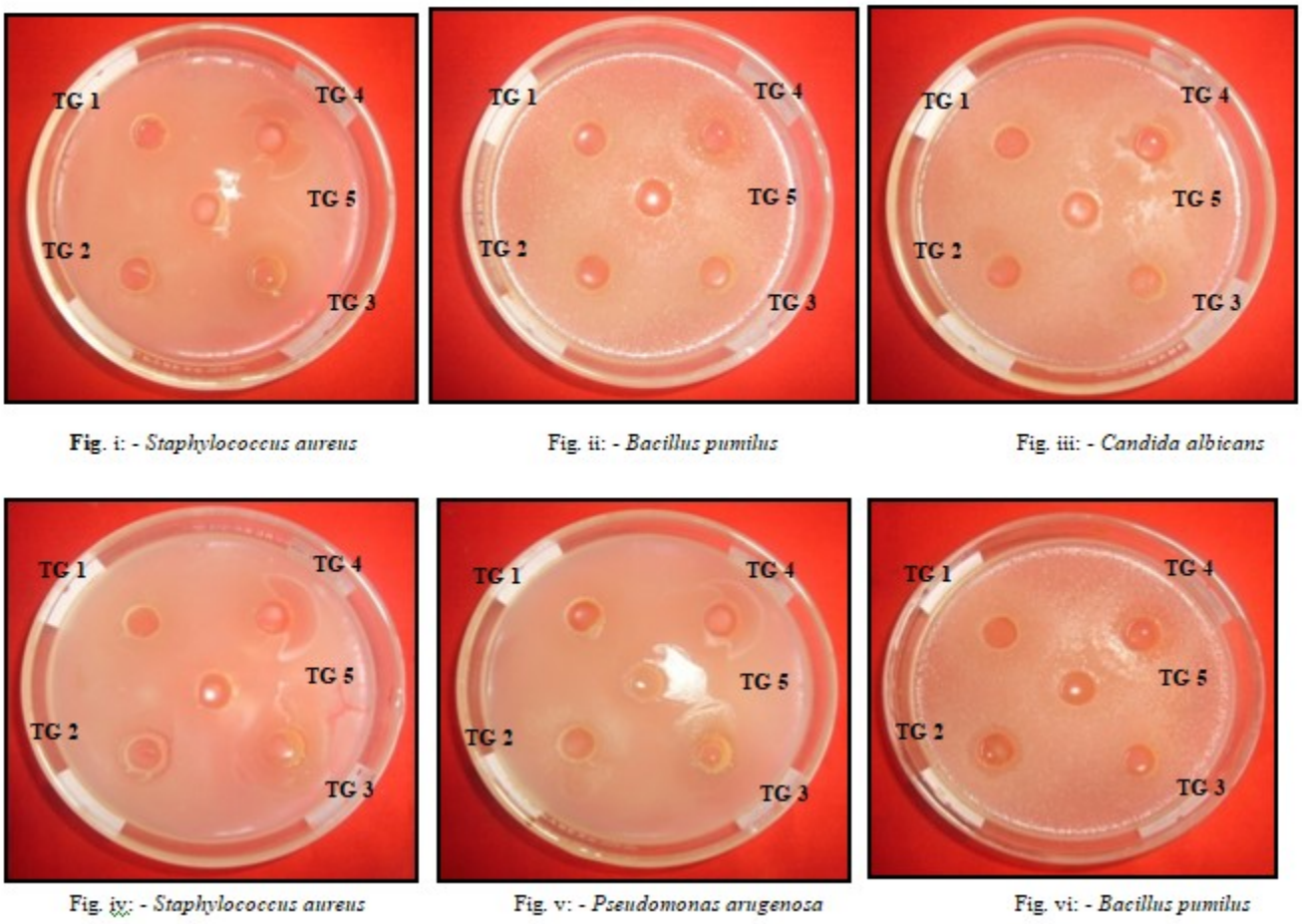


Fig. i: - *Staphylococcus aureus* Fig. ii: - *Bacillus pumilus* Fig. iii: - *Candida albicans*

Fig. iv: - *Staphylococcus aureus* Fig. v: - *Pseudomonas aruginosa* Fig. vi: - *Bacillus pumilus*

Plate 1; (Fig. i – vi):- Antimicrobial activities of actinomycete isolates no. TG 1 – TG 5 (Fig. i – iii):- after 3-day fermentation; (Fig. iv – vi):- after 4-day fermentation; the zone of inhibition was measured using a millimeter scale from the line of agar well as shown in (Table 3, 4 & 5).

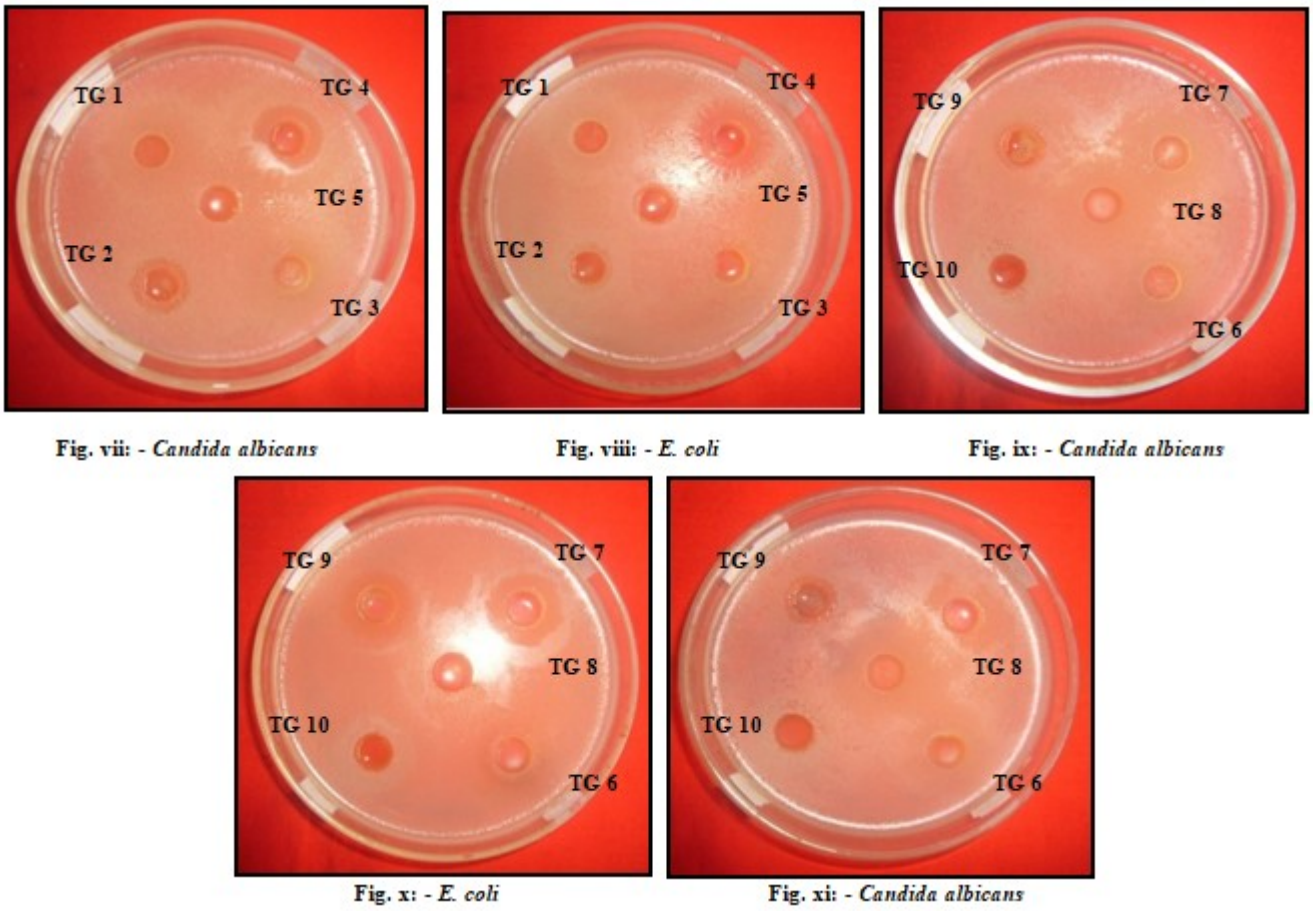


Fig. vii: - *Candida albicans* Fig. viii: - *E. coli* Fig. ix: - *Candida albicans*

Fig. x: - *E. coli* Fig. xi: - *Candida albicans*

Plate 2; (Fig. vii – xi):- Antimicrobial activities of actinomycete isolates no. TG 1- TG 5 (Fig. vii – viii):- after 4-day fermentation; (Fig. ix – xi):- Antimicrobial activities of actinomycete isolates no. TG 6 – TG 10 (Fig. ix):- after 3-day fermentation; (Fig. x):- after 4-day fermentation; (Fig. xi):- after 5-day fermentation; the zone of inhibition was measured using a millimeter scale from the line of agar well as shown in (Table 3, 4 & 5).

Table 3:- ANTIMICROBIAL ACTIVITY OF ACTINOMYCETE STRAINS FOR 72 HOURS (3 DAYS)

Strains No.	Culture Period	Test Organisms					
		Bacillus subtilis	*Staphylococcus aureus*	*Pseudomonas arugenosa*	*Bacillus pumilus*	*Candida albicans*	*E. coli*
TG 1	72 hours 3 days	-	-	12	-	11	-
TG 2		-	12	12	-	12	-
TG 3		-	14	13	-	12	-
TG 4		-	25	18	25	23	17
TG 5		-	12	12	-	-	-
TG 6		-	-	-	-	-	-
TG 7		-	-	-	-	23	-
TG 8		-	-	-	-	24	-
TG 9		12	-	-	-	15	18
TG 10		-	-	17	-	-	-

Table 4:- ANTIMICROBIAL ACTIVITY OF ACTINOMYCETE STRAINS FOR 96 HOURS (4 DAYS)

Strains No.	Culture Period	Test Organisms					
		Bacillus subtilis	*Staphylococcus aureus*	*Pseudomonas arugenosa*	*Bacillus pumilus*	*Candida albicans*	*E. coli*
TG 1	96 hours 4 days	12	12	11	-	11	-
TG 2		12	14	11	14	15	12
TG 3		12	13	11	11	12	-
TG 4		12	24	22	24	24	23
TG 5		11	11	11	-	11	11
TG 6		-	-	-	-	-	-
TG 7		-	-	-	-	-	20
TG 8		-	-	-	-	-	-
TG 9		18	11	-	15	-	18
TG 10		-	-	15	-	-	-

Table 5:- ANTIMICROBIAL ACTIVITY OF ACTINOMYCETE STRAINS FOR 120 HOURS (5 DAYS)

Strains No.	Culture Period	Test Organisms					
		Bacillus subtilis	*Staphylococcus aureus*	*Pseudomonas arugenosa*	*Bacillus pumilus*	*Candida albicans*	*E. coli*
TG 1	120 hours 5 days	12	12	12	12	12	12
TG 2		12	12	12	12	12	12
TG 3		12	12	12	12	12	13
TG 4		12	12	12	18	-	-
TG 5		12	12	12	-	11	12
TG 6		-	-	-	-	-	-
TG 7		-	-	-	-	22	-
TG 8		-	-	-	-	25	-
TG 9		13	-	-	-	14	17
TG 10		-	-	-	-	-	-

Agar well – 10 mm; 10 mm – 14 mm (+); 15 mm – 19 mm (++); 20 mm & above (+++)

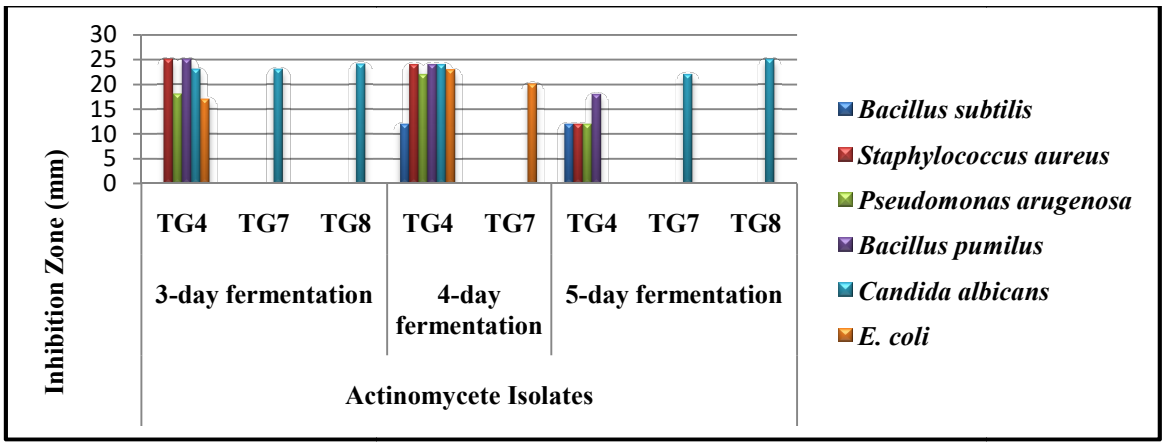


Fig. 2:- Antimicrobial Activities of Actinomycete Strains: No. TG 4, TG 7 & TG 8 after 3-day fermentation, 4-day fermentation and 5-day fermentation. Only the antimicrobial activities of the most active isolates have been shown. The zone of inhibition was measured using a millimeter scale from the line of the agar well as shown in Plate 1 & 2.

After 3-day and 4-day fermentation, isolates no. TG 1, TG 2, TG 3, TG 5, TG 9 and TG 10 showed the moderate antimicrobial activity against the six test microorganisms (Table 3 & 4). Isolate no. TG 6 showed no antimicrobial activity against the six test microorganisms after 3-day, 4-day and 5-day fermentation (Table 3, 4 & 5). After 5-day fermentation, isolates no. TG 1, TG 2, TG 3, TG 5 and TG 9 showed the moderate antimicrobial activity against the six test microorganisms. Isolate no. TG 6 and TG 10 showed no antimicrobial activity against the six test microorganisms (Table 5). During 3-day to 5-day fermentation periods, isolates no. TG 4 showed the broad spectrum of antimicrobial activity and TG 7 and TG 8 exhibited the narrow spectrum of antifungal activity. Moreover, TG 7 showed the narrow antibacterial activity against *E. coli* as shown in Fig. 2.

The results in this study indicated that the findings are in accordance with previous observations described by [5], [7], [8], [10]. The utilization of the selective medium for the preliminary isolation of soil actinomycetes was effective and efficient time-saving. It can be noted that the isolated actinomycetes were going to be identified as specific levels.

IV. CONCLUSION

From this study, it is clearly indicated that the soil samples in Taunggyi area, southern Shan State, Myanmar can provide rich sources of antibiotic producing actinomycetes. These active actinomycetes may be effectively used in large scale production for commercial and pharmaceutical applications in the coming future. The potential of actinomycetes in the discovery of novel compounds with activity against microorganisms has been realized, and hence open exciting avenues in the field of biotechnology and biomedical research.

ACKNOWLEDGMENT

We would like to acknowledge with gratitude to Dr Nwe Nwe Yin, the Acting Rector of Panglong University, Southern Shan State, Myanmar for her permission and administrative support to publish this research journal. We are greatly indebted to Dr Min Min Soe, Professor and Head, Department of Botany, Panglong University for her review of this manuscript. Our sincere thanks are due to our colleagues at Pharmaceutical Research Department, Yangon, for their invaluable guidance, advice, suggestion and comments.

REFERENCES

[1] Atlas, R.M., (2010). *Handbook of Microbiological Media*, 4th ed., p. 637, Washington, D.C., ASM Press.
[2] Conn, H.J., & Conn, J.E., (1941). "Value of Pigmentation in Classifying Actinomycetes. A Preliminary Note." *Journal of Bacteriology*, Vol. 42, pp. 791-800.
[3] Die, K.A., (1914). "Aktinomyceten und ihre Bedeutung in der Natur. Centr." *Journal of Bacteriolog,y*, Vol.41, pp. 649-688.
[4] Harley, J.P., & Prescott, L.M., (2002). *Laboratory Exercises in Microbiology*, 5th ed., p. 139-140, 446, 448, McGraw-Hill Press.
[5] Intra, B., Mungsuntisuk, I., Nihira, T., Igarashi, Y., and Panbangred, W., (2011). "Identification of A,ctinomycetes from Plant Rhizospheric Soils with Inhibitory Activity against *Colletotrichum* spp., the Causative Agent of Anthracnose disease." *BioMed Central Journal*, Vol. 4, pp. 98-102.
[6] Mohan, G.M., and Singara Charya, M.A., (2012). "Enzymatic Activity of Fresh Water Actinomycetes." *International Research Journal of Pharmacy*, Vol. 3, pp. 193-197.
[7] Sarkar, P., Bhagavatula, S., Suneetha, V., (2014). "A Brief Research Study on Novel Antibiotic Producing Isolate from VIT Lake, Vellore, Tamil Nadu." *Journal of Applied Pharmaceutical Science*, Vol. 4, pp. 61-65.
[8] Sharma, M., (2014). "Actinomycetes: Source, Identification and Their Applications." *International Journal of Current Microbiology and Applied Science*, Vol. 3, pp. 801-832.
[9] Singh Singh, S.L., Indra B., and Bora, T.C., (2006). "Actinomycetes of Loktak Habitat: Isolation and Screening for Antimicrobial Activities." *Biotechnology Journal*, Vol. 5, pp. 217-221.
[10] Younis, S.K., Sreekanth, P.M., and Malla, S., (2014). "Screening and Characterization of Putative Antibiotic Compound Using PCR." *European Journal of Experimental Biology*, Vol. 4, pp. 85-92.
[11] Waksman, S.A., (1961). The Actinomycetes, Vol.I: Their Nature, Occurrence, Activities and Importance, 2nd ed., p. 4-11, U.S.A.: Waverly Press.
[12] Waksman, S.A., (1961). The Actinomycetes, Vol.II: Classification, Identification, and Descriptions of Genera and Species, 2nd ed., p.331, U.S.A.: Waverly Press.