ISOLATION AND ANTIMICROBIAL ACTIVITY OF ENDOPHYTIC CTINOMYCETES FROM DIFFERENT PARTS OF FIVE SELECTED PLANTS AND CHARACTERIZATION OF SELECTED *STREPTOMYCES* (TG-16)

Theingi Aung[1], Zar Zar Yin[2] and Kay Thi Mya[3]

Abstract

A total of eighteen endophytic actinomycetes were isolated from leaf, stem, root of five selected plants such as *Millingtonia hortensis* L., *Aloe vera* L., *Barleria strigosa* Willd., *Desmodium triquetrum* (L.)DC. and *Polygonum chinensis* L. from Pathein University Campus from June to August, 2016. This study was carried out at Biotechnological Development and Resources Centre, Pathein University. The isolated strains were designated as TG 1-18. All of the isolated strains were studied their morphological, microscopical characters and Gram staining. The antimicrobial activity of eighteen isolated strains were studied by agar well diffusion method with ten kinds of test organisms. Among them, ten strains showed the antimicrobial activity on *Agrobacterium tumefaciens, Aspergillus paraciticus, Salmonella typhi, Escherichia coli and Saccharomyces cerevisae.* TG-16 showed the highest antimicrobial activity (27.14mm) against *E. coli.* Therefore, TG-16 was selected and characterized by biochemical reactions. The selected strain TG-16 was gram positive with sporophore as hook, open-loop rectinaculum apertum type, spiral spore chain and spore smooth and oval shape. The colony morphology of TG-16 was raised, entire and circular. In the sugar fermentation test of selected strain TG-16, the acid was produced on all studied carbon sources except galatose, lactose, arabinose and positive results to methyl red, nitrate reduction, citrate utilization, catalase, arginine and casein hydrolysis, growth on streak line of potato slice and sodium chloride level at 2.5%(w/v), melanin was not produced but yellow pigment produced in casein hydrolysis medium. According to the results, TG-16 was characterized as the genus *Streptomyces.*

Keywords: Streptomyces, Antimicrobial activity, Biochemical characterization

Introduction

Endophytic bacteria are microorganisms that colonize the internal tissues of the plant without causing any external sign of infection or negative effect on their host (Schulz and Boyle, 2006). Fungi, bacteria or actinomycetes have been found in endophytic association with plants. Endophytic microorganisms can be derived from any part of the plant like bark, leaves, flowers, fruits, roots, seeds etc. Endophytes are an under-investigated group of microorganisms that represent a plentiful and renewable source of bioactive and chemically new compounds with potential for exploitation in a wide variety of medical, agricultural, and industrial realms. Actinomycetes are prokaryotic filamentous, branching bacteria with a fungal type of morphology. The physiological and biochemical characteristics in actinobacterial systematics and identification are still meaningful.

Genus Streptomyces belongs to the Streptomycetaceae family. The filaments and spores are very small usually 1µm or less in diameter. The spores are formed by the fragmentation of the filaments and are borne in straight, wavy, or helical chains. The colonies are slow-growing and often have a soil-like odour because of production of a volatile metabolite, geosmin. They produce a wide variety of pigments responsible for the colour of the vegetative and aerlial mycelia. Streptomyces species are nonmotile, catalase positive, reduce nitrates to nitrites and

[1] Lecturer, Department of Botany, Pathein University
[2] Associate Professor, Department of Botany, Pathein University
[3] Professor and Head, Department of Botany, Pathein University

degrade adenine, esculin, casein, gelatin, hypoxanthine, starch, and L-tyrosine (Hasani *et al*., 2014)

The Actinomycetes also synthesizes and excrete darkpigments, melanin or melanoid, which are considered to be a useful criterion for taxonomical studies. Melanins are frequently used in medicine, pharmacology, and cosmetics preparations (Dastager S.G *et al*.,2006). The aim and objectives of this study were to isolate endophytic actinomycetes from five selected plants and to identify the selected strain TG-16 based on their colony morphology, Gram straining, microscopical characters and biochemical reactions.

Materials and Methods

Study area and collection of plant samples

Millingtonia hortensis L., *Aloe vera* L., *Barleria strigosa* Willd., *Desmodium triquetrum* (L).DC. and *Polygonum chinensis* L. were used to isolate endophytic actinomycetes. The plants samples (leaf, stem, root) were collected from Pathein University Campus, Ayeyarwady Region from June to August, 2016. The identification of these plants were referred by (Flora of Hong Kong, 2009).

Isolation of endophytic microorganisms (Coombs and Franco 2003).

The leaves, stems and roots of selected plant samples were used to analyze. The plant samples were washed by running tap water for several times to remove soil particles and then they were cut into small pieces (0.5 ×0.5cm for leaf and 0.2cm for stem and root) and after that sterilized by sequential immersion in alcohol 70% (1 min), sodium hypochlorite 1.5% (5 min) to eliminate unwanted endophytic fungi or bacteria and finally rinsed with sterile distilled water for three times to remove surface sterilization agents. The surface sterilized plant samples were dried in the folds of sterile paper. After proper drying the surface sterilized plant materials were cultured on four different media, there were Glycerol asparagine agar medium (M-1), Yeast extract malt extract agar medium (M-2), Emerson agar medium (M-3), Starch casein agar medium (M-4) and supplemented with chloramphenicol and nystatin for 7-10 days at room temperature.

Preliminary study of antimicrobial activity of isolated actinomycetes

Eighteen endophytic actinomycetes isolates were inoculated into precultured media respectively (medium-I, Medium-II, Medium-III and Medium-IV) for 4 days at room temperature. After incubation period, the inoculated strain was transferred into the seed medium (M-2) for 3 days at room temperature. After 3 days, then seed medium(25%) was transferred into fermentation medium(Glycerol 0.2%, Peptone 0.5%, Yeast extract 0.3, Malt extract 0.3%, CaCO3 2.5% at pH 7.0) worked out for 3-10 days and evaluated by agar well diffusion method.

Screening of antimicrobial activity by agar well method (Collins 1965)

The assay medium (glucose 1%, Peptone 0.3%, agar 1.8% at pH 7.0) was utilized for test organisms. The molten sterile medium was cooled to 40-45ºC, inoculated with test organism and thoroughly mixed and then, poured into sterile petri plates and allowed to settle. And ten wells were made using sterile cork borer (8 mm in diameter) in the plate and then fermentation broths (20µL) was added to wells . The diameter of inhibition zones around each well were measured and recorded after 24 hrs incubation.

Test organisms

Agrobacterium tumefaciens NITE 09678, *Aspergillus paraciticus* IFO5123, *Bacillus subtilis* IFO 90571, *Candida albicans* NITE 09542, *E.coli* AHU 5436, *Micrococcus luteus* NITE 83297, *Pseudomonas fluorescens* IFO 94307, *Saccharomyces cerevisiae* NITE 83297, *Salmonella typhi* AHU 7943, *Staphylococcus aureus* AHU 8465 were obtained from NITE(National Institute of Technology and Evaluation, Kisarazu, Japan).

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 sugar fermentation of carbohydrate test (Cowan, 1975), Catalase test (Salle, 1948), starch hydrolysis test (soluble starch, rice powder, wheat powder, tapioca powder, sticky rice powder) (Pelezar and Chan, 1972), urea hydrolysis test (Christenson, 1946), potato plug test (Atlas 1993), nitrate reduction test (Harrigan and Mc Cance ,1966), mannitol salt broth test (Marshall 1992), methyl red test, (Bisen and Verma, 1998), citrate test (Atlas, 1993), hydrogen sulfide Test (Cowan, 1975), Voges proskaucer test (Cruickshank, 1963), Hanging slide test, Oxidase test, argenine hydrolysis, gelatin hydrolysis test (Dubey and Maheswari , 2002),casein hydrolysis test (Aneja, 1996),esterace hydrolysis test (Prescott, 2002),melanin production test (Shirling and Gottlieb 1966), salt tolerance test (Atlas, 1993), respectively.

Results

From this study, 18 isolates of endophytic actinomycetes were found. Among them 11 strains were obtained from stem of *Milllingtonia hortensis* L., two strains from stem of *Aloe vera* L., one strain from root of *Barleria strigosa* Willd. each one strains from leaf, stem and root of the *Desmodium triquetrum* (L). DC., and one strain from root of *Polygonium chinensis* L. The aerial mass color of TG-1, 3-6 were white, TG-10-13, 16, 17 were whitish blue while TG-2, 7,8, 9, 14, 15 were greenish white, creamy white, black, pale brown gray, pale blue and pale yellow respectively. The reverse color of TG-1-7, 9, 10 and 16 were pale yellow, TG-11-15 and 17 were pale brown, TG-8 and 18 were black.

The colony size of TG-1-7, 9-11, 13 and 17 were medium while TG-8, TG-14-16 and TG-18 were small and TG-12 was large. The elevation and forms of TG-1, 3, 7, 12, 17-18 were flat, TG-2, 4-6, 8, 14 and15 were convex, TG-10-11,13,16 were raised while TG-9 was umbonate. The elevation and forms of TG-1, 3, 7, 12, 17-18 were flat, TG-2, 4-6, 8, 14and 15 were convex, TG-10-11, 13, 16 were raised while TG-9 was umbonate. The spore chains of all strains generally produce flexibilis, retinaculum and spiral types. The spore chains of TG-1, 2, 9, 11,12, 15, and 17 were flexible type, TG-3,16 showed retinaculum apartum type, TG-4, 5 and 6 were spiral type and TG-8 showed biverticilliate (no spiral) type and isolated strains of TG- 7,10, 13, 14, 18 were short spore chains as shown in Figure (1 and 2).

Eleven isolated strain (TG-2, 3, 4, 5, 7, 9, 12, 13, 15 and 16) had antimicrobial activity and remaining eight isolates could not produce antimicrobial metabolites. Among them, antibacterial activity of isolated actinomycetes TG-16 exhibited the maximum inhibitory zone (27.14 mm) against *Escherichia coli* in 6 days fermentation period. These results were shown in Figure (3). The results of colony morphology, cultural characteristics, microscopical characters and some biochemical tests for the selected strains TG-16 were shown in Table 4-6 and Figure 4-12.

Table 1 Number of endophytic actinomycetes from different parts of five selected plants

Name of plants	Leaf	Stem	Root
Milliongtonia hortensis L.	-	-	TG1, TG-2, TG-3, TG-4, TG-5, TG-6, TG7, TG-8, TG-9, TG-10,TG-11
Aloe vera L.	-	TG-12, TG-13	-
Barleria strigosa Willd.	-	-	TG-14
Desmodium Triquetrum (L). DC.	TG-17	TG-16	TG-15
Polygonum chinensis L.		-	TG-18

Table 2 Colony morphology of the isolated strains

Isolated strain	Size of colony	Margin	Color		Elevation and form
			Surface	Reverse	
TG-1	Medium	Circular	White	Pale	Flat
TG-2	Medium	Circular	Greenish	Pale	Convex
TG-3	Medium	Circular	White	Pale	Flat
TG-4	Medium	Circular	White	Pale	Convex
TG-5	Medium	Circular	White	Pale	Convex
TG-6	Medium	Circular	White	Pale	Convex
TG-7	Medium	Circular	Creamy white	Pale	Flat
TG-8	Small	Circular	Black	Black	Convex
TG-9	Medium	Circular	Pale brown	Pale	Umbonate
TG-10	Medium	Circular	Whitish blue	Pale	Raised
TG-11	Medium	Circular	Whitish blue	Pale	Raised
TG-12	Large	Irregular	Whitish blue	Pale	Flat
TG-13	Medium	Circular	Whitish blue	Pale	Raised
TG-14	Small	Circular	Gray	Pale	Convex
TG-15	Small	Circular	Pale blue	Pale	Convex
TG-16	Small	Circular	Whitish blue	Pale	Raised
TG-17	Medium	Circular	Whitish blue	Pale	Flat
TG-18	Small	Irregular	Pay yellow	Black	Flat

Table 3 Zone of inhibition (in mm) of isolated strain TG-16 on five kinds of test organisms in (4-8) days fermentation peroid

Fermentation peroid	Test organisms									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
4 day	10.73	19.03	-	-	-	11.96	-	23.57	-	10.73
5 day	17.08	18.28	-	-	-	17.68	-	24.96	-	17.64
6 day	15.42	17.13	-	-	-	17.97	-	**27.14**	-	14.34
7 day	14.08	15.73	-	-	-	17.20	-	24.13	-	14.20
8 day	14.11	15.10	-	-	-	17.11	-	23.02	-	14.18

Well size=8mm

1. *Agrobacterium tumefaciens*
2. *Aspergillus paraciticus*
3. *Bacillus subtilis*
4. *Candida albicans*
5. *Micrococcus luteus*
6. *Salmonella typhi*
7. *Pseudomonas fluorescens*
8. *Escherichia coli*
9. *Staphphylococcus aureus*
10. *Saccharomyces cerevisiae*

Table 4 Cultural characteristics of selected strain TG-16 by using ISP- three medium

Medium	Cultural characteristic	
	Surface color	**Reverse color**
Yeast extract malt extract agar (ISP2)	Pale yellow	Yellow
Glycerol asparagine agar (ISP-5)	Pale yellow	Light brown
Peptone yeast ion agar (ISP-6)	Pale yellow	Light bnrown

Table 5 Biochemical test for sugar fermentation of selected strain TG-16

Sugar sources	Responces	Results	
		Acid production	**Gas production**
Sucrose	Yellow color change in medium	+	-
Xylose	Yellow color change in medium	+	-
Fructose	Yellow color change in medium	+	-
Glucose	Yellow color change in medium	+	-
Arabinose	No change in color	-	-
Lactose	No change in color	-	-
Galatose	Yellow color change in medium	+	-
Maltose	No change in color	-	-

Table 6 Biochemical tests for selected strain TG-1

No.	Biochemical Tests	Responses	Result
1	Urea hydrolysis test	No change in pink color	-
2	Mannitol salt broth test	Change red color in test broth	+
3	Nitrate reduction test	Change cherry red color in test broth	+
4	Methyl red test	Medium remain red	+
5	Voges proskaucer test	No change in color of medium	-
6	Citrate utilization	Medium change from green to blue	+
7	H_2S production	No change in color of medium	-
8	Catalase test	Release free oxygen gas bubble	+
9	Oxidase test	No change in color	-
10	Hanging slide test	No motility	-
11	Arginine hydrolysis	Change back to purple from yellow	+
12	Casein hydrolysis	Clear zone around the colony	+
13	Gelatin hydrolysis	No clear zone around the streak line	-
14	Potato plug	Growth on streak line of potato slice	+
15	Melanin production test	No pigmentation in medium	-
16	Starch Hydrolysis		
	(i) Soluble starch	Clear zone around the streak line	++
	(ii) Tapioca powder	Clear zone around the streak line	+
	(iii)Sticky rice powder	Clear zone around the streak line	++
	(iv)Wheat powder	Clear zone around the streak line	++
	(v) Rice	Clear zone around the streak line	+
17	Salt tolerance test		
	(i) 1.5%	Moderate growth	++
	(ii) 2.5%	Highest growth	+++
	(iii)3.5%	Moderate growth	++
	(iv)4.5%	Poor growth	+
	(v) 5.5%	Poor growth	+
	(vi)6.5%	Poor growth	+

positive = +, negative = –, + (poor growth), ++ (moderate growth), +++ (Hightest growth)

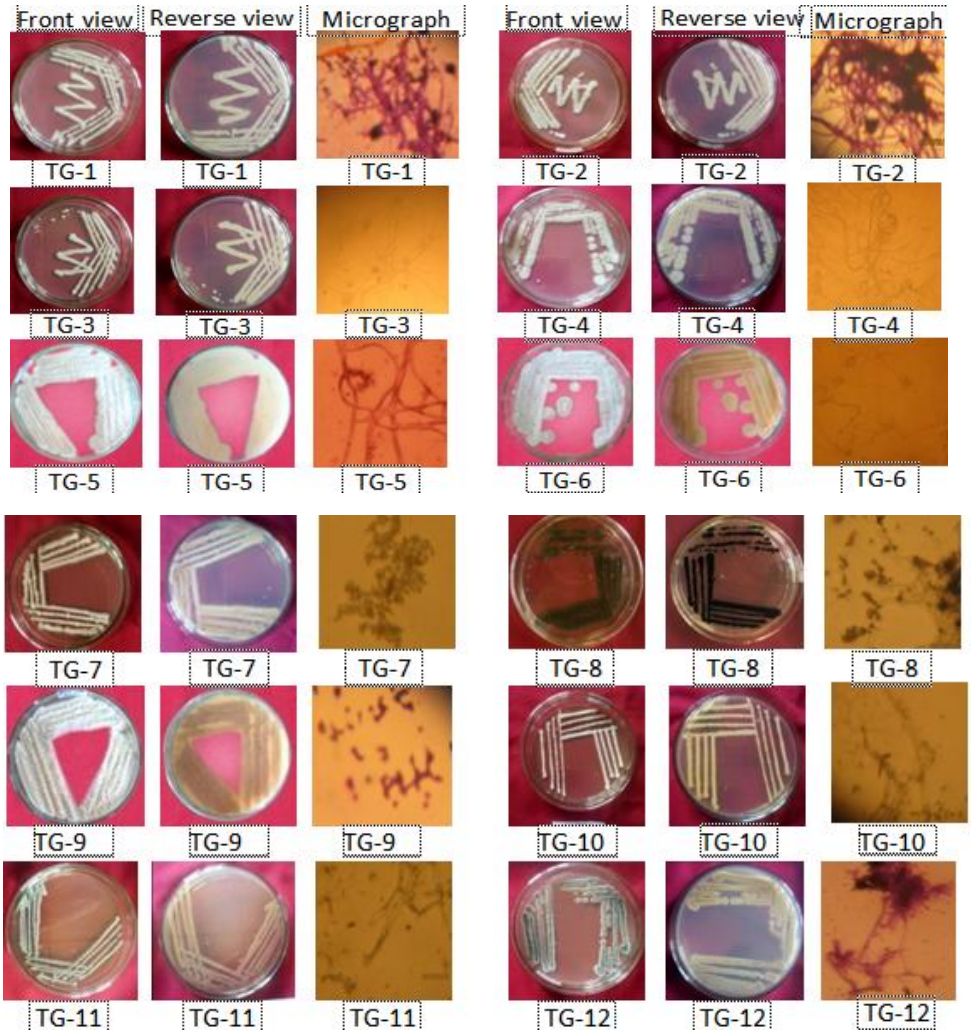


Figure 1 Cultural characteristics (surface α reverse view) and micrograph of isolated strains TG-1 to 12

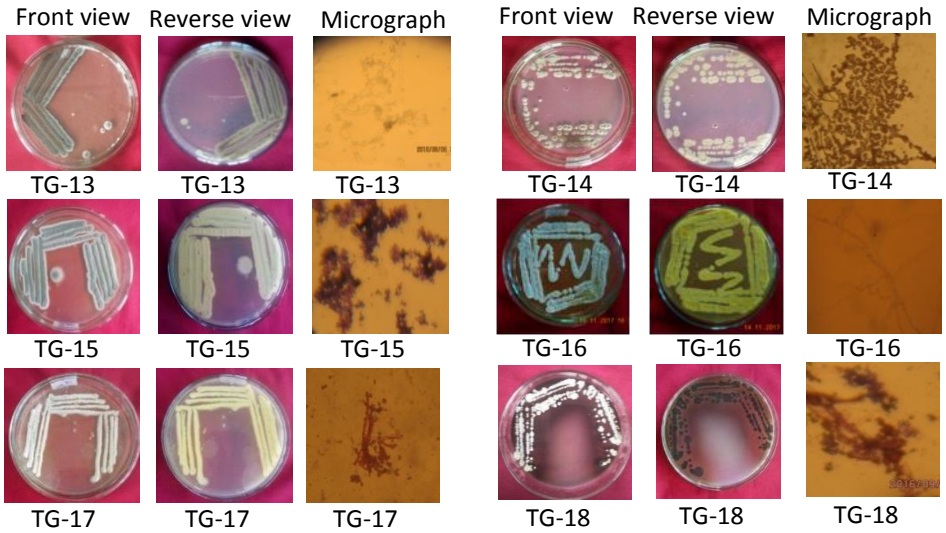


Figure 2 Cultural characteristics (surface α reverse view) and micrograph of isolated strains TG-13 to 18

Figure 3 Antimicrobial activity (in mm) of selected strain TG-16 against *Escherichia coli* at (4-8) days fermentation period

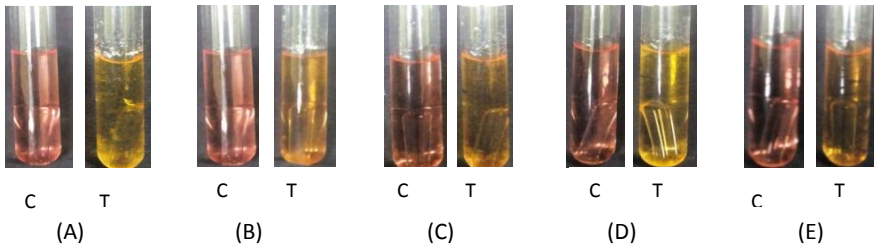


Figure 4 Biochemical test for sugar fermentation of selected strain TG-16
(A) Sucrose (B) Xylose (C) Fructose (D) Glucose (E) Maltose(All positive)

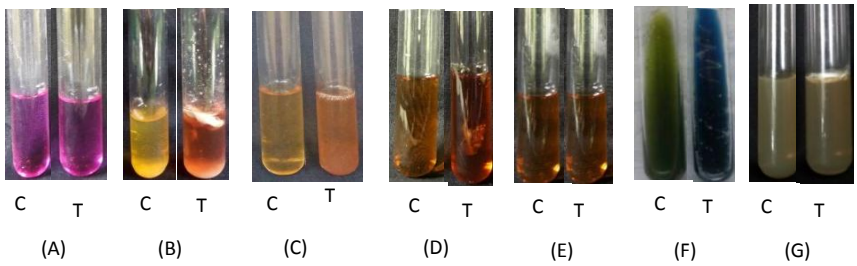


Figure 5 Biochemical test for selected strain TG-16 (A) Urea test (negative) (B)Manitol test (positive) (C) Nitrate reduction test (positive) (D) MR test (positive) (E) VP test (negative) (F) Citrate reduction test (positive) (G) Hytrogen sulphide test (negative)

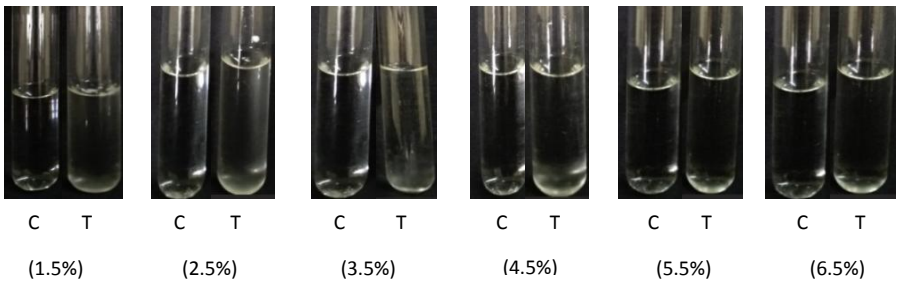


Figure 6 Sodium chloride tolerance of selected strain TG-16 (1.5%-moderate growth), (2.5%-highest growth), (3.5%-moderate growth), (4.5%-poor growth), (5.5%-poor growth) and (6.5%-poor growth)

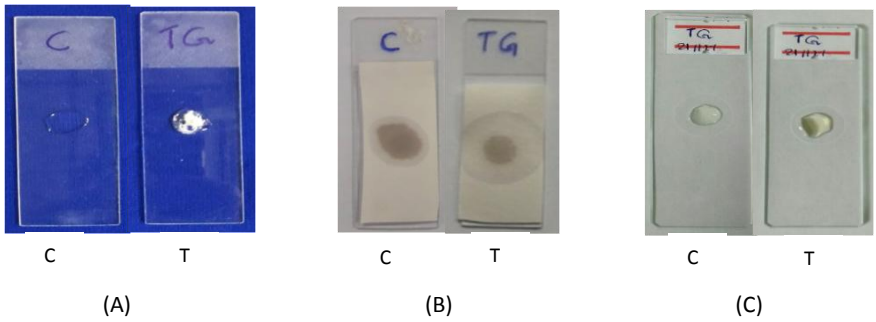


Figure 7 Biochemical test for selected strain TG-16. (A) Catalase test(positive), (B) Oxidase test(negative), (C) Molity test (negative)

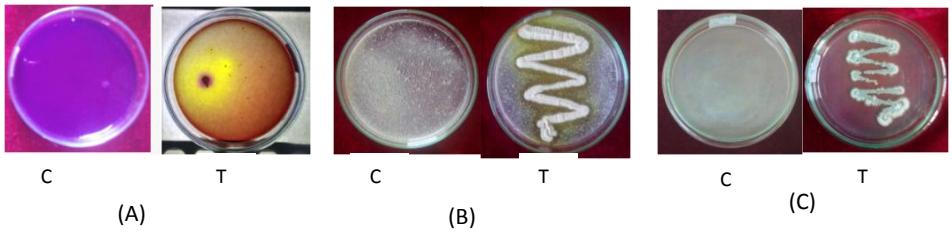


Figure 8 Biochemical test for selected strain TG-16 (A) Arginine hydrolysis (positive) (B) Casein hydrolysis (positive) and (C) Gelatin hydrolysis (negative)

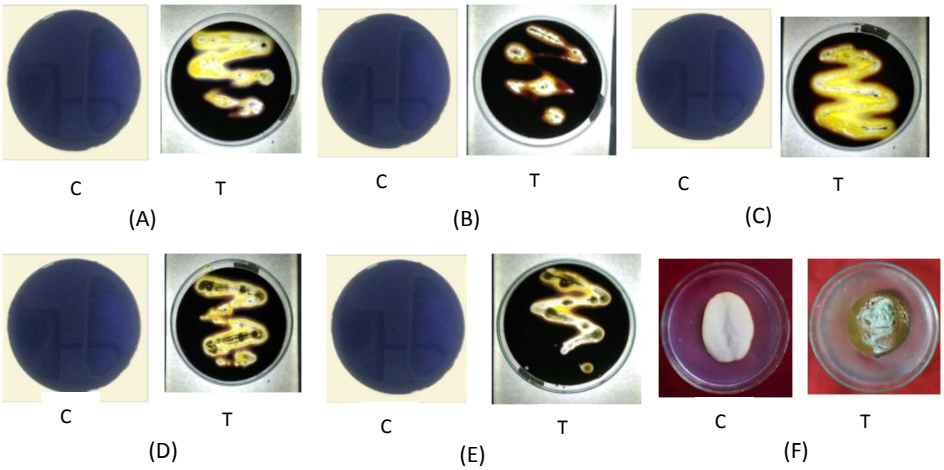


Figure 9 Biochemical test of starch hydrolysis for selected strain TG-16
(A) Soluble Starch (positive) (B) Tapioca powder (positive) (C) Sticky rice (positive) (D) Wheat flour(positive) (E)Rice (positive) and (F) potato plug test (positive)

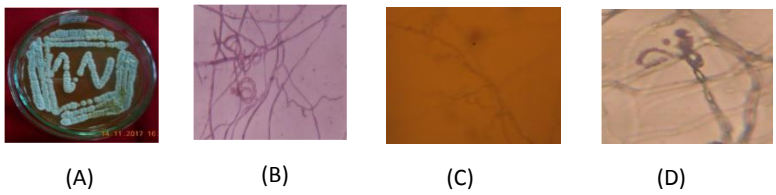
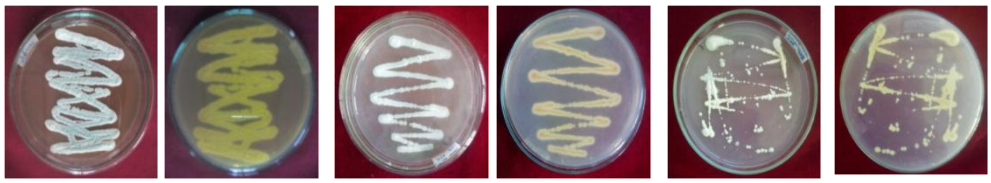
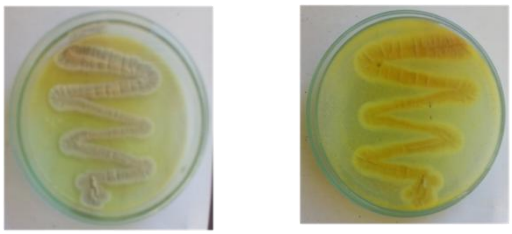


Figure 10 Cultural characteristics and Micrograph of selected strain TG-16
(A) Culture (Streaks Method) (B, C) aerial hyphae with sporophore Retinaculiaparti type (D) sporophore bearing spiralspore chain



Surface (ISP-2) Reverse Surface (ISP-5) Reverse Surface (ISP-6) Reverse

Figure 11 Cultural characteristics of selected strain TG-16 on medium (ISP-2, ISP-5, ISP-6)



Surface view of TG-16 Reverse view of TG-16

Figure 12 Yellow pigment production in casein hydrolysis test medium of TG-16

Discussion and Conclusion

The endophytic actinomycete that resides in the tissue of living plants and does not visibly harm the plants are known as endophytic actinomycetes (Stone *et al.*,2000). In this study, different parts (leave, stem,root) of five selected plant samples were collected from Pathein University Campus, Ayeyarwady Region. Eighteen endophytic actinomycetes were obtained from these plant samples, there were eleven strains from root of *M. hortensis* L., two strains from stem of *A. vera* L., one strain from root of *B, strigosa Willd.*, each one strain from leaf, stem and root of *D. triquetrum* (L).DC. , and one strain from root of *P. chinensis* L.

Out of eighteen isolates, more diverse endophytic actinomycetes were isolated from roots rather than from stem and leaf. Verma *et al*., 2009, recommended that the majority of endophytic actinomycetes have been isolated from root rather than other organs. In this study, eleven strains were obtained from root of *Millingtonia hortinsis* L. Shimizu, 2011 also reported multiple strains of endophytic actinomycetes could be isolated from a single plant.

In order to identify the isolated strains, the colony morphology, gram strains, microscopical characters were carried out. The aerial mass color of all strains were white, gray, whitish gray, whitish blue, dark greenish brown and yellow. The spore chains of all strains were generally produced flexibilis, retinaculum apertum and spiral types. Chatsuda and Kaewalin, 2015 was reported that the type of spore chain of isolated actinomycetes from *Centella asiatica* (L.)Urban. were rectus-flexibils, retinaculum-apertum and spira.

Eleven isolated strain (TG-2, 3, 4, 5, 7, 9, 12, 13, 15 and 16) had antimicrobial activity. Among them, antibacterial activity of isolated actinomycetes TG-16 exhibited the maximum inhibitory zone against *Escherichia coli.* In the study of morphological, microscopical characters and Gram staining reaction, the selected strain TG-16 was gram positive, with sporophore as

hook, open loop retinaculum apertum type. Moreover TG-16 was spiral spore chain and spore smooth and oval shape, elevation, margin and form of these strains were raised, entire and circular.

Three medium; ISP-2, ISP-5 and ISP-6 were used for the cultural characteristics of selected strain TG-16. In this study, aerial mycelium showed pale blue in all medium but reverse color observed yellow in ISP-2, light brown in ISP-5 and ISP-6. The aerial mass color of TG-16 varied from white and pale blue to different nuances of grey (from pale grey to green-grey), therefore it could be a signed to the grey series. These characters was agreement in the result of Stefka, *et al*., 2007. In the study of some biochemical tests, positive in starch hydrolysis and casein hydrolysis. Similar result was described by Venkateswara *et al*. 2015.

The other biochemical characters such as methyl red test, citrate utilization, catalase and arginine hydrolysis and nitrate reduction were positive and dextrose, fructose, lactose and sucrose were positive in acid production test and was also produced yellow pigments on starch casein hydrolysis medium. According to the above results, these characters were in the same with those of Reddy *et al*., 2011. These characters were similar to the investigation on the genus *Streptomyces* by the Actinomycetes (volume I and II) of Selman and Waksman, 1961 and Buchanam, 1964. Based on the obtained results of TG-16 was classified as belonging to the genus *Streptomyces* sp.

Detection and identification of *Streptomyces* are valuable, provides medically important bioactive compounds. The present study concluded that the selected strain belongs to *Streptomyces* sp., which also have diffusible pigment production ability. It can be used for food industries and Pharmaceutical industries as a natural colorant and might be useful in cosmetic industries.

Acknowledgements

I would like to acknowledge to the following persons who have supported for this research work: Dr Si Si Hla Bu, Rector, University of Pathein for providing me an permission to do this work, Dr Nilar Myin and Dr Than Htun Pro-rectors, University of Pathein for their suggestion and advices, Dr Kay Thi Mya, Professor and Head of Botany Department, University of Pathein and Professor Dr Wah Wah Lwin, Department of Botany, University of Pathein for their invaluable suggestions and comments offered in writing this research, Dr. Zar Zar Yin Associate Professor from Department of Botany, University of Pathein for her valuable instructions, critical reviews and suggestions for the successful completion of this research paper.

References

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

Atlas, R.M. (1993). **Microbial media**. Boca Raton Ann Arbor, London Tokyo.ological fundamental

Bisen,P.S and K.Verman,1998. **Handbook of microbiology**.CBS Publishers and Distributors, New Dehli, India.

Buchanan, R.E, N.E. Gibbons, (1974). **Bergey's Mannual of Deternative Bateriology. 8th Edition; Batimor,** The Wiliams and Wilkins Company.Christensen W.B.,. **J. Bacteriol,** 52:461

Collins, CH. (1965). Microbial methods. Butterworth & Co., Publishers Ltd., Lodon.

Cruickshan,R.J.,P.Guguid and R.H.R. Swain, (1963). **Medical microbiology.** 11th ed. The English Book Society and S. Living stone Ltd., London

Chatsuda P. and K. Kaewalin, (2015). **Isolation and Screening for Endophytic Actinomycetes from *Centella asiatica* (L.) Urban**

Coombs JT. and C.M.M. Franco, (2003). **Islation and identification of action bacteria from surface sterilized wheat roots. Appl Environ Microbiol** 69(9): 5603-5608.

Dubey, R.C. and D.K. Maheswari, (2002). Practical Microbiology. S. Chand & Co., New Delhi. Dastager S.G., L.Wen-Jun, A.Dayanand, T.Shu-Kun, T. Xin-Peng,

Y.Xiao, X. Li-Hua,and J. Cheng-Lin (2006). **Seperation, identification and analysis of pigment (melanin) production in Streptomyces.** African Journal of Biotechnology Vol. 5 (8), pp. 1131-1134, 16 .

Hasani A.,A.Kariminik,K.Issazadeh, (2014). **Streptomycetes:Characteristics and TheirAntimicrobial Activities.** International journal of Advanced Biological and Biomedical ResearchVolume 2, Issue 1,: 63-75

Harrigan, W.F. & M.E. Mc Cance. (1996). **Laboratory methods in microbiology academic press inc.,** London.

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.

Reddy N.G, D.P.N. Ramakrishna, S.V. Raja Gopal, (2011)**. A morphological, physiological and Biochemical Studies of Marine Streptomyces rochei (MTCC 10109) Showing Antagonistic Activity Against Selective Human Pathogenic Mcroorganisms**. Asian journal of Biological Sciences.

Salle, A. J. (1948). **Fundamental princioles of bacteriology. Mc.** Graw Hill Book Co., Inc., New York.

Scchulz, B. and C. Boyle, (2006). **Whats are endophytes?** Microbial Root Endophytes, 9:1-13.

Selmam A. and Waksman, (1961). **Classification, Identification of Genera and Species.** The Actinomycetes, Vol. I and II. The Williams & Wilkins Company.

Shimizu, M.,(2011)**. Endophytic actinomycetes: biocontrol agents and growth promoter.** Pp. 201-210

Shirling E.B and Gottlieb D. (1966). **Methods for characterizing Streptomyces sp.** Int J Syst Bacteriol 16:313-340

Stefka A. N., S. Vanya, Y. Lyubomira, (2007). **Taxonomic Study of Streptomyces SP.Strain 34-1**. J. of Culture Collection. Volume 5.pp. 10-5

Stone, J.K., Bacon, C.W., White, J.F., (2000). **An overview of endophytic microbes: endophytism defined.** In: Bacon CW, White JF (eds) Microbialendophytes. Marcel Dekker Inc., New York, pp 3–29.

Kumud Chandra Kandpal, D.A., Umesh Kuma, T. Rashmi, T. Siva Kumar, (2012). **Isolation and screening of endophytic of actinomycetesproducing antibacterial compound from Citrus aurantifolia Fruit.** European Journal of Experimental Biology , 2 (5):1733-1737.

Venkateswara ,VS. and P. Ellaiah (2015)**. A new variant of Streptomyces species–Streptomyces azureusvargossypii from soil of Andhra Pradesh.** Department of Pharmaceutical Sciences Andhra University, IJRPC 5(1), 126-131

Verma J.P.,and J. Yadav, (2009). **Response of wheat to PGPR and organic manures in cereal and legume based cropping sequences under nascent stage of organic farming.** Ann. Plant soil Res., 11: 122-125.