STUDY ON THE FERMENTATION CONDITIONS OF SELECTED ENDOPHYTIC FUNGUS, SL-26 FROM *PRUNUS PERSICA* (L.) BATSCH

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Abstract

The plant samples were collected from Mogok Township, Mandalay Region. These selected plants were identified as Rosaceae family - *Pyrus communis* L. (Thit-taw), *Eriolobus indica* Schnied. (Makhauk), *Prunus armeniaca* L. *(*Met-man), *Prunus persica* (L.) Batsch (Met-mon), and *Cydonia oblonga* Mill. (Chin-shaw-khar), during June to August, 2017. Isolation of endophytic fungi was done by surface sterilization procedure. The antimicrobial activity of these fungi was performed by agar well diffusion method with six kinds of test organisms. Ten fungal strains showed antimicrobial activity. Among them, three fungal strains exhibited the antimicrobial activity on four test organisms. Especially, SL-26 exhibited the moderate antibacterial activity (22.61 mm) against *Escherichia coli* at 5 days. Therefore, SL-26 was selected and the fermentation parameters of this fungus were optimized by the proper age and size of inoculum, effect of carbon and nitrogen sources on antibacterial metabolites production against *E coli*. According to the results, 120 hours age and 25% inoculum size were the most suitable condition for SL-26. In the effect of maximum antibacterial activity on various carbon and nitrogen sources, SL-26 showed the best activity on lactose and asparagine. the comparison of shaking culture and static culture, the antibacterial activity of static culture was more than that of shaking culture. And then the effect of temperature and pH were studied and the best activity was found at 30℃ and pH 6.5. Moreover, thirteen kinds of fermentation media (FM) and fermentation period were investigated. The highest antibacterial activity of SL-26 was obtained in FM-11 and the 5th days to be optimum fermentation incubation period. Endophytic fungi are the group of organism with very good potential for application in plant improvement and disease control.

Keywords: Endophytic fungi**,** antimicrobial activity, fermentation

Introduction

Endophytes are microorganisms in living plant tissues, apparently without inflicting negative effects (Carroll, 1989). Endophytes are presumably ubiquitous in the plant kingdom, some of which can improve the ecological adaptability of hosts (Miller, 2002).

Endophytic fungi are found in all kinds of plants, i.e. trees, grasses, algae and herbaceous plants. These microorganisms could improve the level of resistance to disease and abiotic stress, as well as favoring the growth of crop plants (Waller *et al*., 2005), producing bioactive substances (Lin *et al.,* 2010, Selim *et al*., 2011) and exhibiting antagonistic (Rocha *et al*., 2011) and antimicrobial activity (Kharwar *et al*., 2010).

Endophytic fungi form the promising source for the production of novel products with biological activity (Pimentel *et al.,* 2011). But the production of these metabolites is largely influenced by the nutritional as well as environmental factors associated with the fungi. The nutritional factors include carbon source, nitrogen source etc. and environmental factors include pH, temperature, incubation period (Thakur *et al.,* 2009). Optimization of such factors is necessary for obtaining highest yield of secondary metabolites. The aim and objectives of this research were to isolate the endophytic fungi from five selected plants of Rosaceae family, to evaluate the antimicrobial activity of isolated endophytic fungi, to investigate the optimum age and size of inoculum, to screen the effect of carbon and nitrogen sources, to compare the

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antibacterial activity on shaking culture and static culture, to optimize the effect of pH and temperature on bioactive metabolites production and to observe the effect of sutible synthetic fermentation medium of selected endophytic fungus (SL-26).

Materials and Methods

Study Area and Collection of Plant Samples

These plant samples, *Pyrus communis* L. (Thit-taw), *Eriolobus indica* Schnied. (Makhauk), *Prunus armeniaca* L. *(*Met-man), *Prunus persica* (L.) Batsch (Met-mon), and *Cydonia oblonga* Mill. (Chin-shaw-khar) were collected from Mogok Township, Mandalay Region from June to August, 2017. The identification of these plants were referred by (Flora of Hong Kong, 2009 and Hundley and Chit Ko Ko, 1987) at Department of Botany, Pathein University.

Isolation of Endophytic Fungi (Espinosa *et al*., 1991)

In the isolation procedure of endophytic fungi, the leaves were washed in running tap water for 15 minutes and were cut into about 0.3 cm pieces. Then, these parts were sterilized by soaking in 95% ethanol for 15 minutes. And again, these parts were cut into smaller pieces and dried on sterilized tissue paper. After drying these pieces were placed on as Malt Extract Agar (BMEA) medium, Czapek-Dox Agar (CZA) medium, Malt Extract Agar (MEA) medium, Glucose Ammonium Nitrate Agar (GAN) medium, Dichloran-Rose Bengal-Chloramphenicol Agar (DRBC) medium and Potato Dextrose Agar (PDA) medium plate and incubated at room temperature. When hypal tips grow out, they were transferred into Malt Extract Agar (BMEA) medium.

Screening for Antimicrobial Activity (NITE 2005)

The isolated fungi were grown on BMEA medium at room temperature for 5 days. After incubation period, these fungi inoculated into the seed medium (glucose 0.5 g, peptone 0.3 g, yeast extract 0.3 g, $MgSO_47H_2O$ 0.01 g, K_2HPO_4 0.01 g, $CaCO_3$ 0.01 g, DW 100 mL at pH 6.5) for 3 days at room temperature. After three days, the seed medium (25%) was transferred into the fermentation medium (glucose 1.0 g, peptone 0.5 g, yeast extract 0.5 g, $MgSO_47H_2O$ 0.01 g, $K_2HPO_4$0.01 g, $CaCO_3$0.01 g, DW 100 mL at pH 6.5) and carried out for 3- 10days and evaluated the antimicrobial activity by agar well diffusion method.

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

1 day old culture test broth (0.2 mL) was added to 25 mL warm assay medium (glucose 1.0 g, peptone 0.3 g, KNO_3 0.1 g, DW 100 mL, agar 1.8 g) and thoroughly mixed and poured into plate. After solidification, the agar was left to set Cork borer was used to make the wells (8 mm in diameter). And then, the fermented broth (20 µL) was carefully added into the well and incubated at room temperature for 24-48 hours. The diameter of the zones of inhibition around each well measured and recorded after 24-48 hours incubation.

Test Organisms

Escherichia coli AHU 5436, *Bacillus subtilis* IFO 90571, *Bacillus pumilus*, *Candida albicans* NITE 09542, *Pseudomonas fluorescens*, IFO 94307 *Staphylococcus aureus* AHU 8465 were obtained from NITE (National Institute of Technology and Evaluation, Kisarazu, Japan).

Study on the Effects of Ages of Inoculum on Fermentation (Cruger, 1989)

The selected fungus SL-26 was grown on BMEA medium at room temperature for 5 days and then was transferred into seed medium. Incubation period 3 to 10 days were used for the production of antibacterial metabolite and the procedure of seed culture medium was also used as the previous method. And then, seed culture was transferred to 100 mL conical flask containing of fermentation medium and incubated at room temperature. The inoculum age of fermentation were studied by 48, 60, 72, 84, 96, 108, 120, and 132 hr.

Study on the Effects of Sizes of Inoculum on Fermentation (Cruger, 1989)

The selected fungus SL-26 was grown on BMEA medium for 5 days at room temperature. After 5 days incubation period, this fungus was inoculated into 100mL seed medium. For the size of inoculum, seed culture (5%, 10%, 15%, 20%, 25%, 30% and 35%) were transferred into the each flask of 100 mL fermentation medium. All fermentation media were carried out 5 days and antibacterial activity was investigated by agar well diffusion method.

Effect of Carbon Sources

Carbon sources (each 1.0 g or 1.0 mL) such as lactose, glycerol, dextrose, D-mannitol, arabinose, fructose, glucose, maltose, tapioca powder, sucrose, rice, soluble starch, molasses, oat, corn, carrot and potato were used. Fermentation were incubated at room temperature for 5 days.

Effect of Nitrogen Sources

Nitrogen sources (each 0.05 g or 0.05 mL) such as asparagine, malt extract, peptone, fishcake, milk, yeast extract, gelatin, soybean, casein, sodium nitrate, meat, urea, ammonium nitrate, potassium nitrate, rice bran, peanut, ammonium chloride and ammonium sulphate were also used. Fermentation medium were incubated at room temperature for 5 days.

Comparision of Static Culture and Shaking Culture (Hassan *et al*., 2017)

250 mL conical flask containing 100 mL of the fermentation medium was incubated on the shaker (100 rpm) for 5 days. At the same time, another those fermentation medium was incubated under static condition without shaking. These shaking culture and static culture were compared by using agar well diffusion method.

Effect of Incubation Temperature (Cazar *et al*., 2004)

The optimization temperature for antibacterial metabolite production was carried out at six different incubation temperatures viz. 20, 25, 30, 35, and 40 and 45°C. The fermentation medium was carried out 5 days and antifungal activity was studied by agar well diffusion method.

Effect of pH (Furtado *et al*., 2005)

The optimization of pH of the fermentation broth for antibacterial metabolite production was done by carrying out the fermentation at seven different pH values viz. 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0. For each pH value, desired pH by using either 0.1M NaOH or 0.1M HCl was adjusted into fermentation medium

Effect of Different Carbon and Nitrogen on Fermentation Medium

In this study five different of carbon such as lactose, glycerol, D-Mannitol, arabinose and fructose and five different of nitrogen were used such as asparagine, malt extract, peptone,

fishcake and milk. Fermentation medium FM1- (lactose and Asparagine), FM2- (lactose and malt extract), FM3- (lactose and peptone), FM4- (lactose and fishcake), FM5 (lactose and milk), were used as well as, FM6-(Asparagine and Glycerol) FM7-(asparagine and D-Mannitol), FM8-(asparagine and arabinose), FM9-(asparagine and fructose), FM10-(lactose, glycerol and asparagine), FM11-(lactose, glycerol, and malt extract), FM12- (lactose, asparagine and malt extract) and FM13-(glycerol, asparagine and malt extract) were applied.

Media Used in Fermentation (NITE, 2005)

Fermentation was undertaken by the suitable conditions of 25% sizes and 120 hrs ages of inoculum with thirteen different media. Fermentation was carried out for 5 days and antibacterial activity test was carried out 24 hrs.

Effect on Fermentation Period of SL-26

The optimal fermentation parameters period of isolated fungus SL-26 was studied such as 72 hour seed culture, 25% inoculum size, temperature 30˚C, pH-6.5 and static culture of fermentation, the antibacterial activity against *E. coli* was observed 3 days and 10days.

Results

Isolation of Endophytic Fungi

A total of thirty three fungi were isolated from five selected species of Rosaceae family. Isolated fungi were designated as SL. Six isolates (SL-1 to 6) were obtained from *Pyrus communis* L. and the other strains (SL-7 to 17) were isolated from *Eriolobus indica* Schnied. Another strains (SL-18 to 24) were obtained from *Prunus armeniaca* L. and SL-25 to 28 were isolated from *Pyunus persica* (L.) Batsch and (SL-29 to SL-33) were obtained from *Cydonia oblonga* Mill. These results were shown in Table 1 and Figure 1

Table 1 Isolated endophytic fungi

Scientific Name	Myanmar Name	English Name	Fungi
***Pyrus communis* L.**	Thit-Taw	Pear	SL-1 to SL-6
***Eriolobus indica* Schnied.**	Mat-Khauk	Crabapple	SL-7 to SL-17
***Prunus armeniaca* L.**	Met-man	Apricot	SL-18 to SL-24
***Pyunus persica* (L.) Batsch**	Met-mon	Peach	SL-25 to SL_28
***Cydonia oblonga* Mill.**	Chin-shaw-khar	Quince fruit	SL-29 to SL-33

Table 2 Morphological colour of isolatedc Endophytic fungi

No.	Isolated fungi	Surface colour	Reverse colour
1	SL-1	White	White
2	SL-2	Black	Yellow
3	SL-3	Pale brown	Yellow
4	SL-4	Black	White
5	SL-5	Brown	Pale brown
6	SL-6	Pale green	Yellow
7	SL-7	Brown in the center, White at the end	Yellow
8	SL-8	Black	Pale Yellow
9	SL-9	Black	Yellow
10	SL-10	Brown	Brown

No.	Isolated fungi	Surface colour	Reverse colour
11	SL-11	Pale orange	Red
12	SL-12	White	Pale Yellow
13	SL-13	Dark brown	Yellow
14	SL-14	White	White
15	SL-15	Pale brown	White
16	SL-16	Green	White
17	SL-17	Pale yellow	Yellow
18	SL-18	Green in the center, White at the end	Green
19	SL-19	Green	Yellow
20	SL-20	Cream	Yellow
21	SL-21	Brown	Pale Yellow
22	SL-22	Yellow	Cream
23	SL-23	Pale brown	Yellow
24	SL-24	Yellow	Pale Yellow
25	SL-25	Black	White
26	SL-26	Blue green	Cream
27	SL-27	Yellow	Yellow
28	SL-28	Pale green	Yellow
29	SL-29	Pale brown	Black
30	SL-30	Brown	Yellow
31	SL-31	Dark green Brown	Brown
32	SL-32	Cream	Pale brown
33	SL-33	White	White

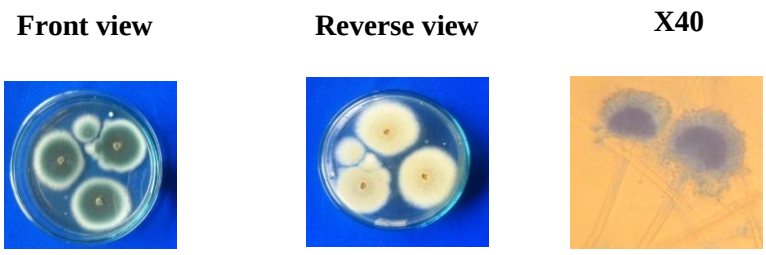

Figure 1 Morphology and microscopical character of selected fungus SL-26

Antimicrobial Activity of Endophytic Fungal Strains

Three endophytic fungi were tested for antimicrobial activity with four test organisms. Agar well diffusion method were employed for assay performance. Three isolated fungal strains exhibited the morderate antimicrobial activity. Among potent strains, SL-26 showed strong antibacterial activity against *Escherichia coli* (22.61mm) in 5 days fermentation period, than other fungal strains SL-6 and SL-19. Therefore, SL-26 was selected for further research.

Table 3 Antimicrobial Activity of Endophytic Fungus SL-26

Fermentation period (days)	**Test organisms and antimicrobial activity (mm)**			
	1	**2**	**3**	**4**
3	15.05	16.22	15.67	16.13
4	18.81	16.40	17.89	17.11
5	22.61	18.02	20.27	17.22
6	-	15.92	17.12	17.43

Antimicrobia; activity of isolated fungi against
(1) *Escherichia coli* (2) *Bacillus subtilis*
(3) *Bacillus pumilus* (4) *Candida albicans*

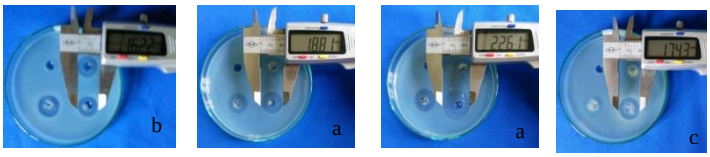

Figure 2 Antimicrobia; activity of isolated fungi against
(a) *Escherichia coli* (b) *Bacillus subtilis*
(c) *Bacillus pumilus* (d) *Candida albicans*

The Effect on Age of Inoculums of SL-26

In the effect of age of inoculum, the antibacterial activity of SL-26 was investigated by using 48, 60, 72, 84, 96, 108, 120, and 132 hrs old culture age of inoculums on *E. coli*. The results showed that 120 hrs age of inoculum gave the highest activities (20.51mm) followed by 20.11 mm at 108 hrs and 19.60 mm at 132 hrs age of inoculum.

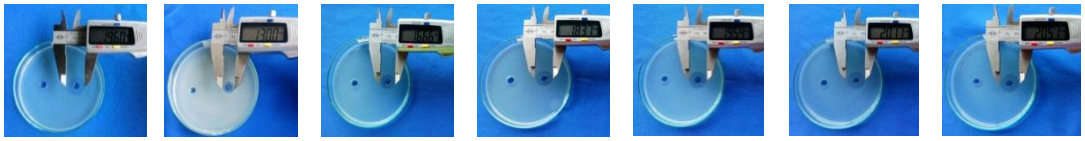

Figure 3 The effect on age of inoculum of SL-26 on *E. coli*

The Fffect on Sizes of Inoculum of SL-26 on *E. coli*

In this research work, the effect of size of inoculum was studied by using 5%, 10%, 15%, 20%, 25%,30% and 35 % inoculum. The highest antibacterial activity was obtained by using 25% inoculum (23.23mm), followed by 20% (23.10mm) and 15% (22.91 mm) respectively.

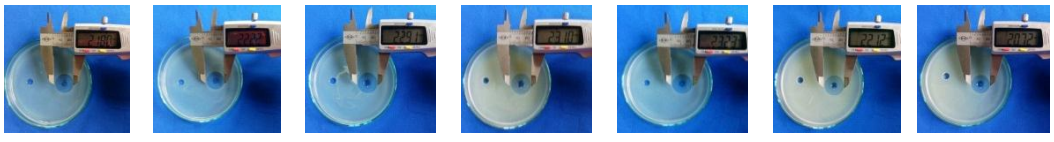

Figure 4 The effect on size of inoculum of SL-26 on *E. coli*

Effect of Carbon and Nitrogen Utilization on the Antibacterial Activity

The significant inhibition zone (22.14 mm and 21.53 mm) were obtained in glycerol and lactose as amended media. The moderate inhibition zone were found on D-mannitol (20.56 mm), arabinose (20.51 mm), fructose (20.39 mm), glucose (20.30 mm), and maltose (20.26 mm). Tapioca powder (19.69 mm), sucrose (19.55 mm), rice (19.23 mm), soluble starch (18.88 mm), molasses (18.56 mm), oat (17.78 mm), corn (17.41 mm), carrot (16.87 mm) and potato (15.58 mm) were regarded as poor inhibition zone. Similarly, the addition of asparagine displayed the greatest activity (23.65 mm), followed by malt extract (23.05 mm), peptone (22.46 mm), fish cake (21.61 mm), milk (21.31 mm), yeast extract (20.62 mm), gelatin (20.48 mm), soybean (20.33 mm), casein (19.94 mm), sodium nitrate (19.80 mm), meat extract (19.42 mm), urea (19.40 mm), ammonium nitrate (18.99 mm), potassium nitrate (18.28 mm), rice bran (17.67 mm) and peanut (17.62 mm). There were no activities when NH_4Cl and $(NH_4)_2SO_4$ were used as nitrogen source. These results were shown in Figure 5 and 6.

Table 4 Effect of carbon and nitrogen utilization on fermentation

Sr. No	Carbon sources	Antibacterial Activity(mm)
1	Lactose	22.14
2	Glycerol	21.53
3	D-Mannitol	20.56
4	Arabinose	20.51
5	Fructose	20.39
6	Glucose	20.30
7	Maltose	20.26
8	Tapioca powder	19.69
9	Sucrose	19.55
10	Rice	19.23
11	Soluble starch	18.88
12	Molasses	18.56
13	Oat	17.78
14	Corn	17.41
15	Carrot	16.87
16	Potato	15.58

Sr. No	Nitrogen sources	Antibacterial Activity (mm)
1	Asparagine	23.65
2	Malt extract	23.05
3	Peptone	22.46
4	Fishcake	21.61
5	Milk	21.31
6	Yeast extract	20.62
7	Gelatin	20.48
8	Soy- bean	20.33
9	Casein	19.94
10	Sodium nitrate	19.80
11	Meat extract	19.42
12	Urea	19.40
13	Ammonium nitrate	18.99
14	Potassium nitrate	18.28
15	Rice bran	17.67
16	Peanut	17.62
17	Ammonium chloride	No activity
18	Ammonium sulphate	No activity

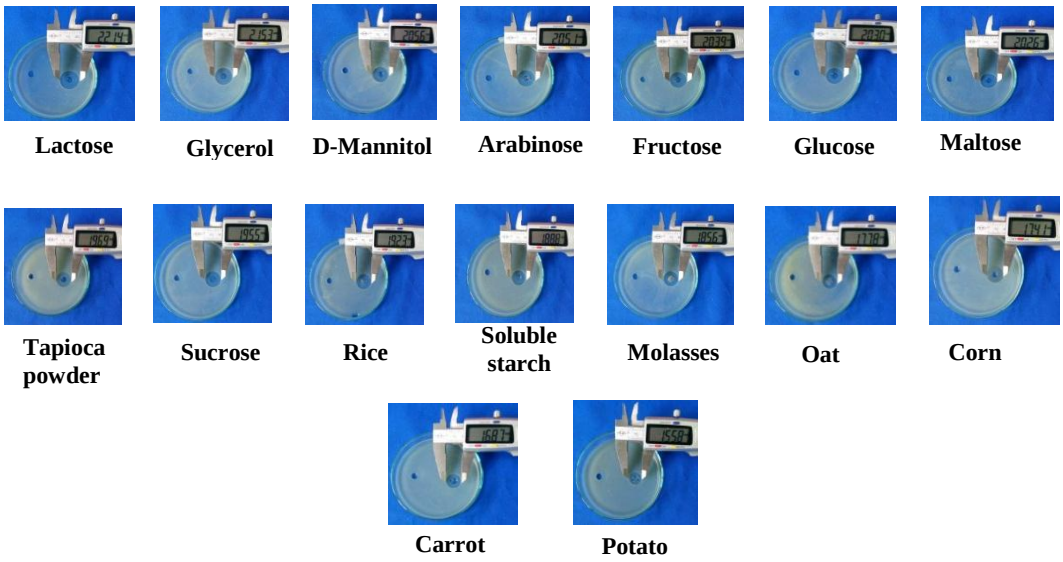

Figure 5 Effectof carbon utilization on the antibacterial activity of SL-26

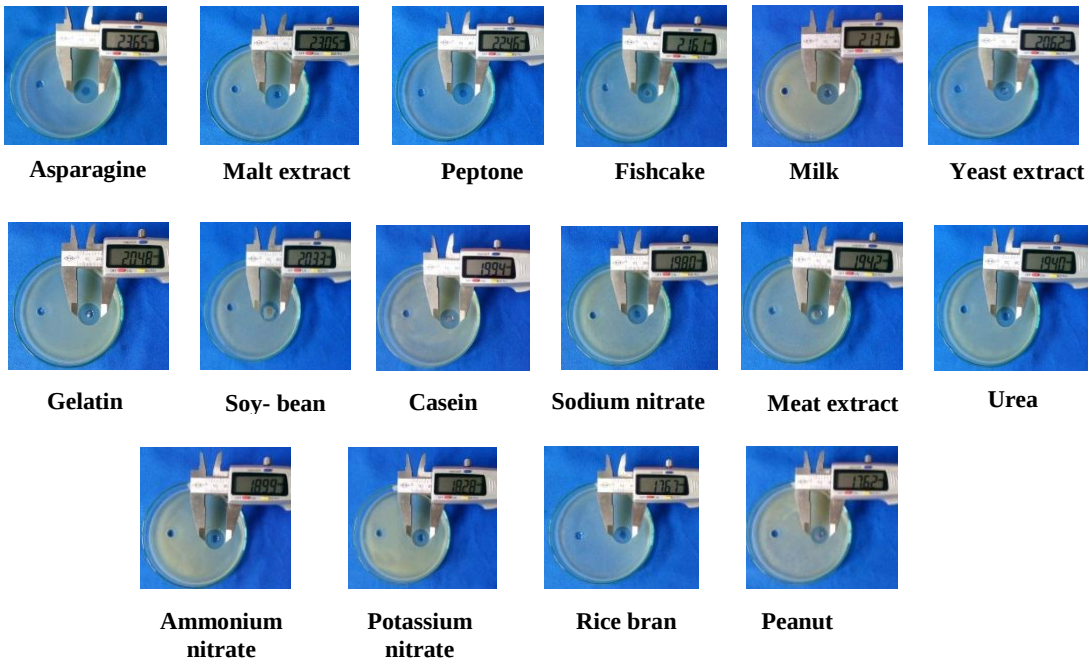

Figure 6 Effect of nitrogen utilization on the antibacterial activity of SL-26

Comparision of Static culture and Shaking Culture

When comparing the static culture and shaking culture of fermentation medium, antifungal activity from static culture is better than (20.52 mm) than that of shaking culture (18.30 mm).

Figure 7 Comparison on static culture and shaking culture of SL-26

Effect of Temperature and pH

Maximum antibacterial activity was recorded at 30˚C (20.59mm), followed by 35˚C (18.76 mm) and 25˚C (17.28 mm). There was a gradual decrease in antibacterial activity when the temperature was increased from 35˚C to 40˚C. Maximum inhibitory zone was occurred in pH 6.5 (20.76mm), followed by pH 7 (20.58 mm), pH 6 (19.89 mm) and pH 7.5 (19.87 mm). Under this conditions, minimum inhibitory zone was observed at pH-5 (17.15 mm), pH-4.5 (15.71mm) and pH-4 (14.98mm) respectively.

Table 5 Effects of different temperature and pH on the antibacterial against *E. coli*

Temperature range	Inhibition zone (mm)	pH range	Inhibition zone (mm)
20ºC	16.53	4	14.98
25ºC	17.28	4.5	15.71
30ºC	**20.59**	5	17.15
35ºC	18.76	5.5	18.53
40ºC	14.10	6.0	19.89
45ºC	13.57	6.5	20.76
		7	20.58
	Static culture	7.5	19.87
		8.0	18.35

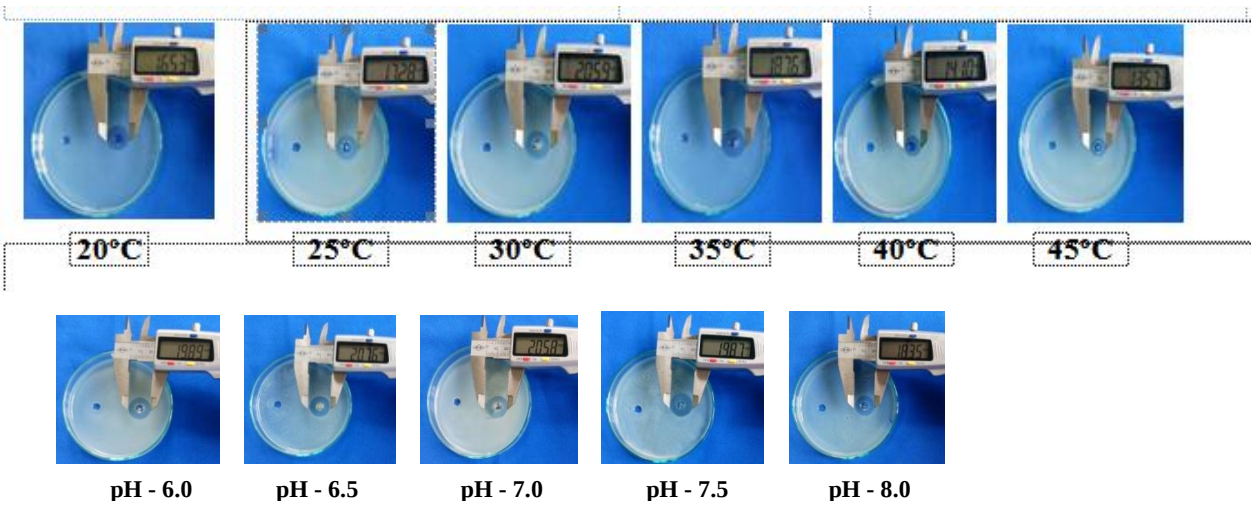

Figure 8 Effects of different temperature and pH on the antibacterial against *E. coli*

Antibacterial Activity of SL-26 on Thirteen Fermentation medium

In this study, FM-11 showed remarkable result (24.15mm) followed by FM-4 (23.76 mm) , FM-10 (23.25 mm), FM-8 (23.18mm) and FM-6 (23.16 mm) were also studied.

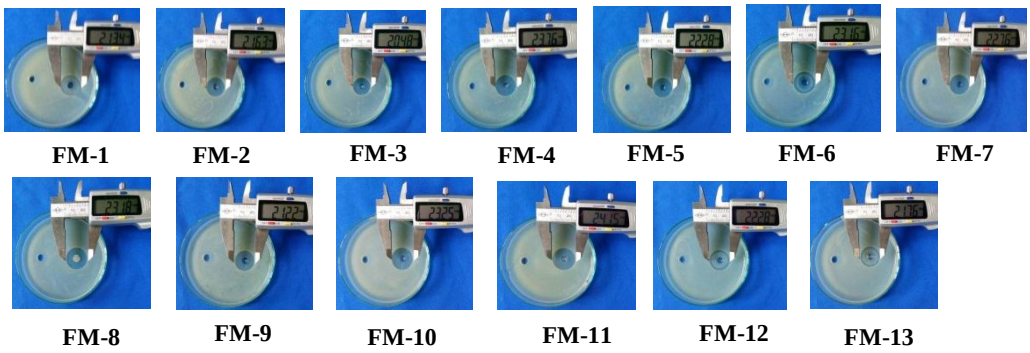

Figure 9 Antibacterial activity of SL-26 on thirteen fermentation medium

Effect of Fermentation Period of SL-26

The optimal fermentation parameters such as 72 hour seed culture, 25% inoculum size, temperature 30˚C, pH-6.5 and static culture of fermentation, the antibacterial activity against E. coli was observed 3 days and 10days. In 5 days fermentation period, the highest antibacterial metabolite of SL- 26 was obtained (26.12mm). And there was gradually decrease antibacterial activity in 6 days to 10 days fermentation period (24.77 mm, 22.76 mm, 20.49mm, 18.71mm and 18.68 mm) respectively.

Table 6 Antibacterial activity on suitable fermentation medium against *E.coli*

Fermentation period	Inhibitory zone
3	19.90
4	20.98
5	26.12
6	24.77
7	22.76
8	20.49
9	18.71
10	18.68

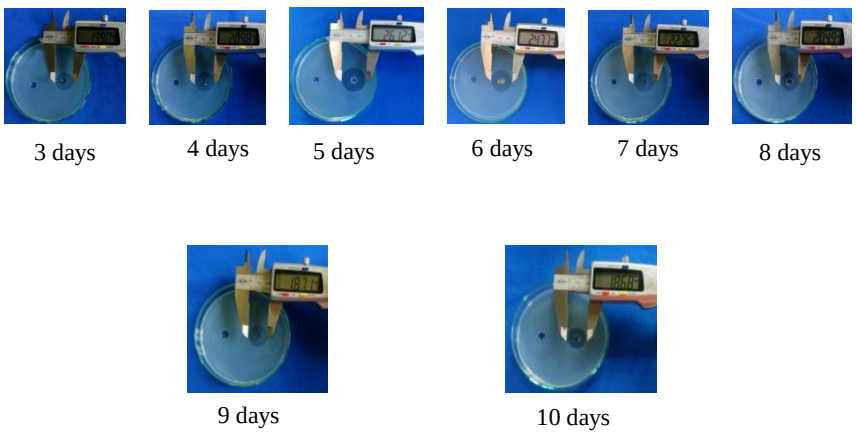

Figure 10 Antibacterial activity on suitable fermentation medium against E.coli

Discussion and Conclusion

The numerous species of fungal endophytes made an ecological niche in the inner space of plants. These ubiquitous fungi interact positively with their environment. Isolation of endophytic fungi from medicinal and other plant results to produce bioactive compound which has greater activity against various pathogenic microbes (Crueger, 1989).

The isolated fungi (SL-6, 19 and 26) could display the antimicrobial activity inhibiting the four test organisms. Among the potent strains, antibacterial activity of isolated fungus SL-26, isolated from *Pyunus persica* (L.) Batsch showed the maximum inhibitory zone of 22.61 mm against *E.coli* in 5 days fermentation period. Similarily, Catherin, 2007, demonstrated that highest zone of inhibition from endophytes of *Camellia sinensis* was observed on *E. coli* and *Staphylococcus aureus*.

Modifying fermentation parameters such as time, temperature, pH, and nutrients can help expanding the range of secondary metabolites (Pfefferle *et al*., 2000). In determining the most suitable size for production antibacterial compounds, 25% inoculum size reached the highest activity (23.23 mm) so that 25% size of inoculum regarded as the most suitable size. In investigation of the effect of carbon, maximum inhibition zone reached up (22.14 mm and 21.53 mm) in lactose and glycerol. Mao *et al.,* 2005 reported the effects of various carbon sources like lactose, sucrose, glucose, fructose, glactose, maltose and xylose. The maximum production of antibacterial metabolite in SL-26 was observed in the presence of asparagine and malt extract (23.65mm and 23.05mm) as nitrogen source. Pimentel *et al*., 2011, reported that the maximum antibacterial activity was obtained when media was supplement with asparagine.

Maximum antibacterial activity was found at pH 6.5 as the diameter of zone of inhibition was 20.76 mm. Maximum inhibitory activity was recorded at the incubation temperature of 30˚C (20.59mm). Compaore 2016, reported that the temperature 30ºC and pH-6.5 were observed to be optimum temperature and pH for antimicrobial activity. According to these results,30˚C is the most suitable temperature for the antimicrobial metabolite production. In this study, under static shaking culture, the diameter of inhibitory zone was more higher than under shaking culture. Fermentation medium (FM-11) was showed significant result by using lactose, glycerol and malt extract. Therefore FM-11 was chosen as a selected fermentation medium.

It was concluded that the present study was to observe the antibacterial activity of three isolated fungi and to investigate the optimum fermentation conditions of selected fungus SL-26 against *E. co*li.

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References

Alberts, J. F., Gelderblom, W. C. A., Thiel, P. G., Marasas, W. F. O., Vanschalkwyk, D. J. and Behrend, Y**.** (1990).**Effects of Temperature and Incubation Period on Production of Fumonisin B1 by *Fusarium moniliforme*. Applied and Environmental Microbiology**, 56 (6): 1729-1733.

Ando , K ., M. Suto and S. Inaba,(2004) . **Sampling and isolating methods of fungi**, Workshop at University of Pathein.

Catherine A.F. (2007). Tr**opical Biomedicine**, 22(2), 165-170.

Carroll G, (1988). **Fungal endophytes in stems and leaves: from latent pathogens to mutualistic symbiont.** Eco; 69: 2-9.

Cazar, M. E., Schmeda-Hirschmann, G. and Astrudillo, L., (2004). **Antimicrobial Butyrolactone I Derivatives from the Ecuadorian Soil Fungus *Aspergillus terreus* Thorn. Var *terreus*.** World J. Microbiol. Biotechnol. 21: 1067-1075.

Crueger, W., & Crueger, A. (1989): **Methods of fermentation**, **in Biotechnology**, **A Textbook of Industrial Microbiology, Internal Student Edition**.; 64-74

Espinosa –Garcia , F . J. and J.H. Langenhein .(1991). **The endophytic fungal community in leaves .** New Phyto. 116: 89 – 97.

Furtado, N. A. J. C, Fonseca, M. J. V. and Bastos, J., (2005). **The potential of an *Aspergillus fumigatus* Brazilian strain to produce antimicrobial secondary metabolites**. Braz. J. Microbiol. 36: 357-362.

Hassan, S. A. A., Bakhiet & S. E. A., (2017). **Optimization of antibacterial compounds production by A*spergillus fumigatus* isolated from sudanese indigenous soil**. Autumn, Vol 3, No 4.

Hundley , H.G. and Chit Ko Ko, (1987). **List of trees, Shrubs, Herbs and Principal Climbers,** etc, 4 ed., Swe Daw Oo Press, Yangon Myanmar.

Kress, J., *et al.,* (2003). **A checklist of the Tree, Shrubs, Herbs and Climbers of Myanmar**, Department of Systematic Biology Botany- National Museum of Natural History, Washington DC, USA.

Mao, X.B, Eksriwong, T., Chauvatcharin, S. and Zhong, j. j. (2005). **Optimization of carbon source and carbon and nitrogen ratio for cordycepin production by submerged cultivation of medicinal mushroom *Cordyceps militaris*.** Process biochem 40:1667-1672.

Miller JD, Mackenzie S, Foto M, Adams GW, Findlay JA (2002). **Needles of white spruce inoculated with rugulosin-producing endophytes contain srugulosin reducing spruce budworm growth rate.** Mycol Res; 106: 471-479.

NITE (National Institute of Technology and Evaluation) (2004). **Media for fermentation to produce the metabolites**.

Pandy, B.P., (1994). **Angiosperms.** S. Chand & Company L.TD. Rm Nagar, New Delhi.Hu Qi- ming *et. Al*., 2008. **Flora of Hong Kong.** Vol II agriculture, Fisheries and Conservation Department Government of the Hong Kong Special Administrative Region.

Petrini, O. (1986). **Taxonomy of endophytic fungi of aerial plant tissue**. In: Fokkema NJ, van den Huevel J, eds. Microbiology of the phyllosphere. Cambridge, UK: Cambridge University Press, 175-187.

Peighamy-ashnaei S, Sharifi-Tehrani A, Ahmadzadeh N, Behboudi K, (2007). **Commin. Agric**. *Appl. Biol. Sci*., 72: 951-6.

Pfefferle ,C., Theobald, U. and Gurtler, H., (2000). **Improved secondary metabolite production in the genus *Streptosporangium* by optimization of the fermentation conditions**. J Biotechnol. 80:135-42.

Pimentel, M.R., Recco, M., Molina, G., Dion´ýsio, P., ostica Juniorm M.R. and Pastore, G. (2011). **The use of endophytes to obtain bioactive compounds and their application in biotransformation process** SAGE-Hindawi Access to Research. *Biotechnol. Res. Int.* Article ID 576286. 11

Rocha ACS, Garcia D, Ueanabaro APT, Carneiro RTO, Araujo IS, Mattos GRR, Goes- Neto A. (2011).- **Foliar endophytic fungi from *Hevea brasiliensis* and their antagonism on *Microcyclus ulei*.** Fungal Diversity 47, 75-84.

Sarika R. Deshmukh, Yogita K. Dhas and B.A.Patil, (2014). **Comparative account on medicinal importance of *Momordica charantia* and its endophytes**.Volume3, Issue 9, 632-640.

Selim KA, El-Bein AA, Abd El-Rahman TM, EL-Diwany AI. (2011). **Biodiversity and antimicrobial activity of endophytes associated with Egyptian medicinal plants.** Mycosphere 2, 669-678.

Thakur, DB, Bora, TC, Bordoloi, GN and Mazumdar, S. (2009). **Influence of nutrition and culturing conditions for optimum growth and antimicrobial metabolite production by *Streptomyces*** sp. 201. J Med Mycol, 19:161-167.