Study on the Fermentation Conditions of Selected Soil Fungus against *Escherichia coli* and *Candida albicans*

Tin May Htwe[1]

Abstract

The present study was focused on the fermentation conditions of selected soil fungus on *Escherichia coli* and *Candida albicans.* The selected soil fungus was used for the investigation of optimal fermentation conditions such as age and size of inoculum, various carbon and nitrogen sources, various fermentation medium. In the age and size of inoculum, the selected fungus showed the highest antimicrobial activity at 120 hrs old culture and 25% inoculum size. In utilization of carbon sources, maltose was the best whereas yeast extract was the best in nitrogen sources. In the fermentation medium (FM), this fungus gave the highest antimicrobial activity at FM 6 (22.11 mm and 16.64 mm) followed by FM 12 (20.97 mm and 16.79 mm) and FM 10 (20.30 mm and 16.32 mm) on *E.coli* and *C. albicans*. These results indicated that the selected soil fungus may be utilized by the optimal fermentation conditions for the screening of antimicrobial activity.

Keywords: size of inoculum, age of inoculum, fermentation medium

Introduction

There is a huge variation in the time for emergence of resistance, which varies among organisms and antibiotics. At this point, a new antibiotic is requires, which is active against resistant bacteria (Luzhetsky *et al.*, 2007). Many organisms can use a single organic compound to supply both carbon and energy needs. Following the carbon sources, the nitrogen sources are generally the next most plentiful substance in the fermentation media. A few organisms can also use the nitrogen source as the energy source (Rhodes and Fletcher, 1996). Nitrogen can be inorganic such as ammonium salts, or organic such as amino acids, proteins and urea. The carbon substrate has a dual role in biosynthesis and energy generation, with carbohydrates being the usual carbon source for microbial fermentation processes (Stanbury *et al.*, 1995). Fungi may not have evolved regulation on acid production due to the availability of excess sugars in their environment for metabolism; thereby when grown on synthetic media with available carbohydrates, fungi proceed to convert sugar to acid which subsequently leads to the cessation of growth (Magnuson *et al.*, 2004). The aim and objectives of this research were to investigate the age and size of selected fungus, to study the effects of carbon and nitrogen sources and to observe the fermentation medium.

Materials and Methods

Agar Well Method (Collins, 1965)

Isolated strains were tested by agar well method for the preliminary antimicrobial activities. Cork borer was used to make the wells (8 mm in diameter) in the autoclaved basal medium for antimicrobial test. Wells impregnated with 3-6 days old culture fermented broth (20 μL) were incubated at room temperature for 24-48 hours. After 24-48 hours of incubation, the clear zones were measured. Therefore, the diameter of clear zones had been observed as potent activity as shown by respective strain. Clear zones surrounding the wells indicated the presence of antimicrobial activities which inhibit the growth of the test organisms selectively.

[1] Vice-Principal, Yankin Education Degree College

Table 1. Eight kinds of Test Organisms used for Antimicrobial Activity (NITE and PRD)

No	Test Organisms	Diseases
1	*Escherichia coli*	Diarrhoea, pneumonia, abdominal pain
2	*Bacillus pumilus*	Fever
3	*Bacillus subtilis*	Fever
4	*Candida albicans*	Candidasis, skin disease
5	*Pseudomonas fluorescens*	Rice disease
6	*Staphylococcus aureus*	Boil and food poisoning
7	*Agrobacterium tumefaciens*	Plants disease, Crown gall disease
8	*Malassezia furfur*	Danddruff, Seborrhoeic dermatitis

Age and Size of inoculums for selected fungus

In the study of age of inoculums, the incubation of seed culture times- 48 hrs, 72 hrs, 96 hrs, 120 hrs, 144 hrs and 168 hrs were used and transferred into the fermentation media. Fermentation was carried out for seven days and antimicrobial activity was tested by agar well diffusion method. In the investigation of size of inoculums, 5%, 10%, 15%, 20%, 25% and 30% were used for the antimicrobial activity of selected fungus. Seed culture was incubated at room temperature.

Utilization of carbon and nitrogen sources

In this study, the morphological characters of strain were studied by using various carbon and nitrogen sources. To determine the effect of antimicrobial metabolite production of different carbon sources such as carrot, corn, dextrose, fructose, glucose, lactose, maltose, oak, potato, rice, soluble starch, starch, sucrose, topioca and xylose were used. Nitrogen sources such as ammonium chloride, ammonium nitrate, ammonium sulphate, casein, gelatin, malt extract, beef extract, milk, peanut, peptone, potassium nitrate, sodium nitrate, urea and yeast extract were used.

Antimicrobial activity of selected strain on various fermentation Media

The biosynthesis of secondary metabolites greatly was affected by medium composition. Fermentation was undertaken with suitable condition of 120 hrs age of inoculum and 25% size of inoculum with fifteen different media. Fermentation was carried out 120 hrs and antimicrobial activity was carried out every 24-48 hrs.

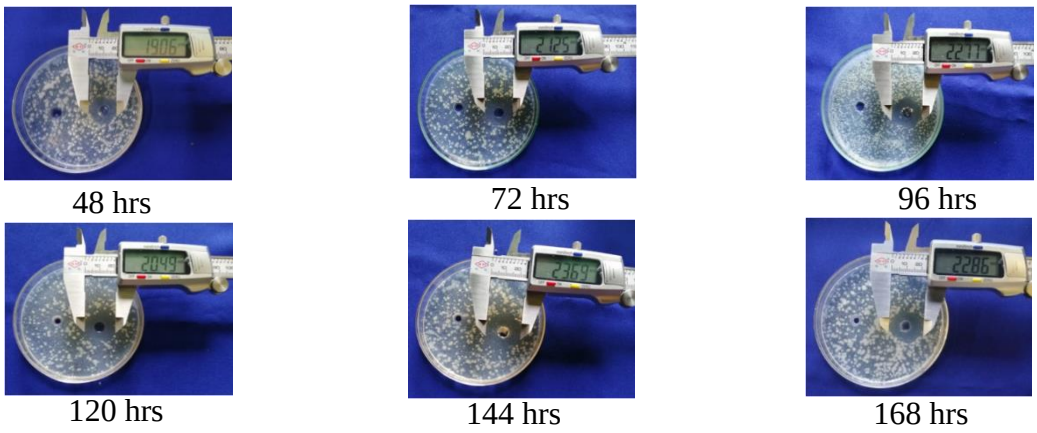
Results

Effect of ages of inoculums on the fermentation of selected strain

In the effect of ages of inoculums, selected strain was investigated by using 48, 72, 96, 120, 144 and 168 hours old culture ages of inoculum. The results showed that 120 hrs ages of inoculums gave the highest activities (23.69 mm) and (23.02 mm) against *Escherichia coli* and *Candida albicans* followed by 144 hrs (22.86 mm and 20.08 mm), 96 hrs (21.77 mm and 21.22 mm), 72 hrs (21.25 mm and 20.49 mm) and 168 hrs (20.22 mm and 19.90 mm) against *Escherichia coli* and *Candida albicans.* These results have shown in Table (2) and Figure (1).

Table 2. The effect of age of inoculum of selected fungus against *Escherichia coli and Candida albicans*

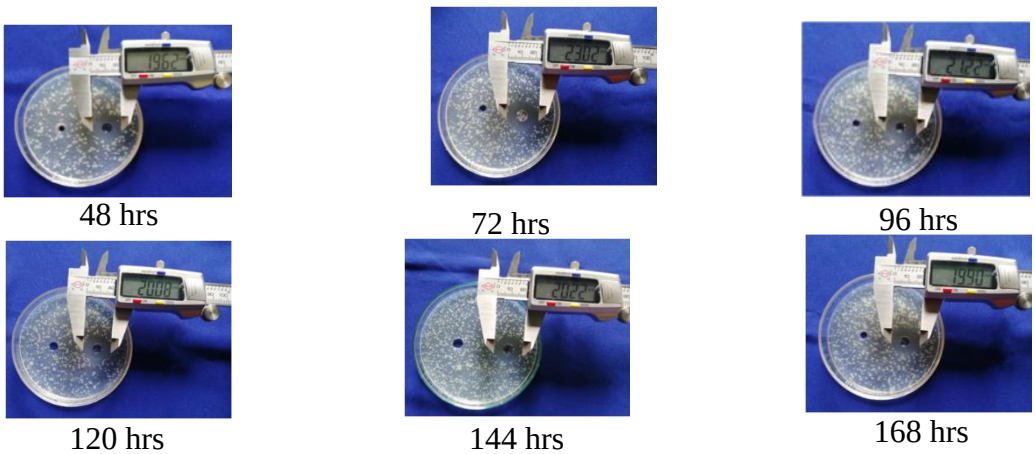
No	Ages of inoculums (hrs)	Two test organisms and Inhibition Zone(mm)	
		Escherichia coli	***Candida albicans***
1	48	19.06	19.62
2	72	21.25	20.49
3	96	22.77	21.22
4	**120**	**23.69**	**23.02**
5	144	22.86	20.08
6	168	20.22	19.90



48 hrs 72 hrs 96 hrs

120 hrs 144 hrs 168 hrs

Fig. 1 (a). The effect of ages of inoculums of selected fungus against *Escherichia coli*



48 hrs 72 hrs 96 hrs

120 hrs 144 hrs 168 hrs

Fig. 1 (b). The effect of ages of inoculums of selected fungus against *Candida albicans*

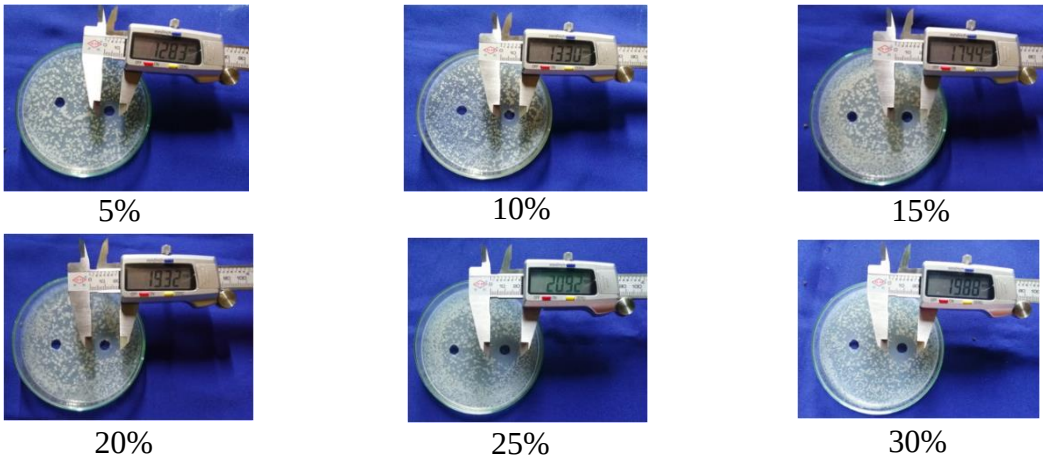
Effect of sizes of inoculums on the fermentation of selected strain

In this research work, the effect of sizes of inoculums were studied by using 5%, 10%, 15%, 20%, 25% and 30% inoculums. When using 25% inoculums of fermentation, TM-14 showed higher activities 20.92 mm and 19.57 mm than 30%

(19.88 mm and 19.20 mm), 20% (19.32 mm and 17.77 mm), 15% (17.44 mm and 17.25 mm), 10% (13.30 mm and 15.13 mm) and 5% (12.83 mm and 15.73 mm) against *Escherichia coli and Candida albicans.* These results have shown in Table (3) and Figure (2).

Table 3. The effect of size of inoculums of selected strain against *Escherichia coli* and *Candida albicans*

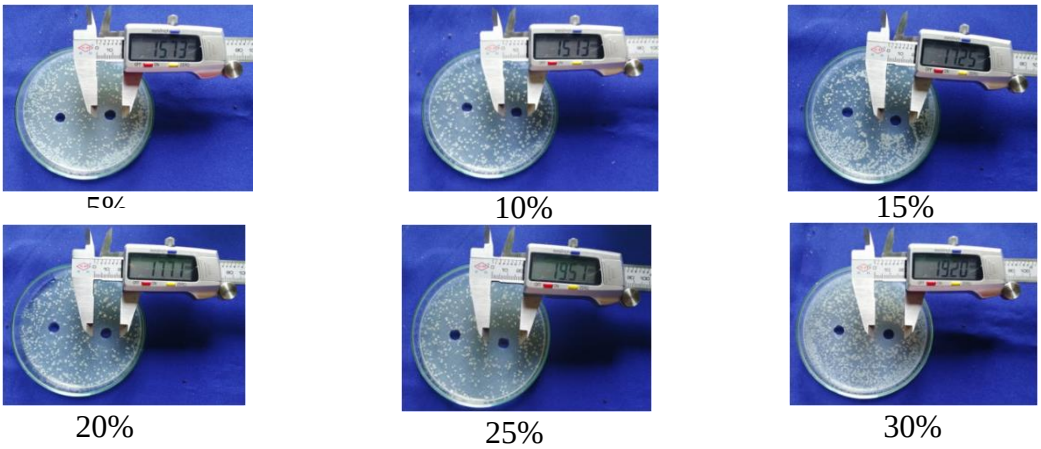
No	Size of inoculums (%)	Two test organisms and Inhibition Zone(mm)	
		Escherichia coli	***Candida albicans***
1	5	12.83	15.73
2	10	13.30	15.13
3	15	17.44	17.25
4	20	19.32	17.77
5	**25**	**20.92**	**19.57**
6	30	19.88	19.20



5% 10% 15%

20% 25% 30%

Fig. 2 (a). The effect of size of inoculums of selected fungus against *Escherichia coli*



10% 15%

20% 25% 30%

Fig. 2 (b). The effect of size of inculums of selected fungus against *Candida albicans*

Effect of carbon sources on antimicrobial metabolite production of fungus

In this study, the biosynthesis of secondary metabolites greatly affected by medium compositions. The addition of Maltose resulted maximum activity and inhibition zone reached (26.52 mm and 24.76 mm), followed by sucrose (25.67 mm and 24.69 mm), lactose (23.26 mm and 22.86 mm) and fructose (23.46 mm and 22.19 mm) against *Escherichia coli* and *Candida albicans.* These results have shown in Table 4 and Figure 3.

Table 4. The Effect of Carbon Source on the Fermentation of selected strain against *Escherichia coli* and *Candida albicans*

Sr. No	Carbon Source	Two test organisms and Inhibition Zone (mm)	
		Escherichia coli	***Candida albicans***
1	Carrot	19.52	15.38
2	Corn	17.23	17.74
3	Fructose	23.46	22.19
4	Glucose	19.71	18.66
5	Lactose	23.26	22.86
6	Sucrose	25.67	24.69
7	Oak	19.53	19.22
8	Potatose	22.17	20.30
9	Rice	17.42	16.81
10	Soluble Starch	23.18	22.57
11	Xylose	22.27	20.58
12	Topioca	21.08	17.82
13	Maltose	**26.52**	**24.76**

12 -14 mm=weak activity, 15 - 19 mm = high activity, >20 mm=very high activity (agar well size = 8 mm)

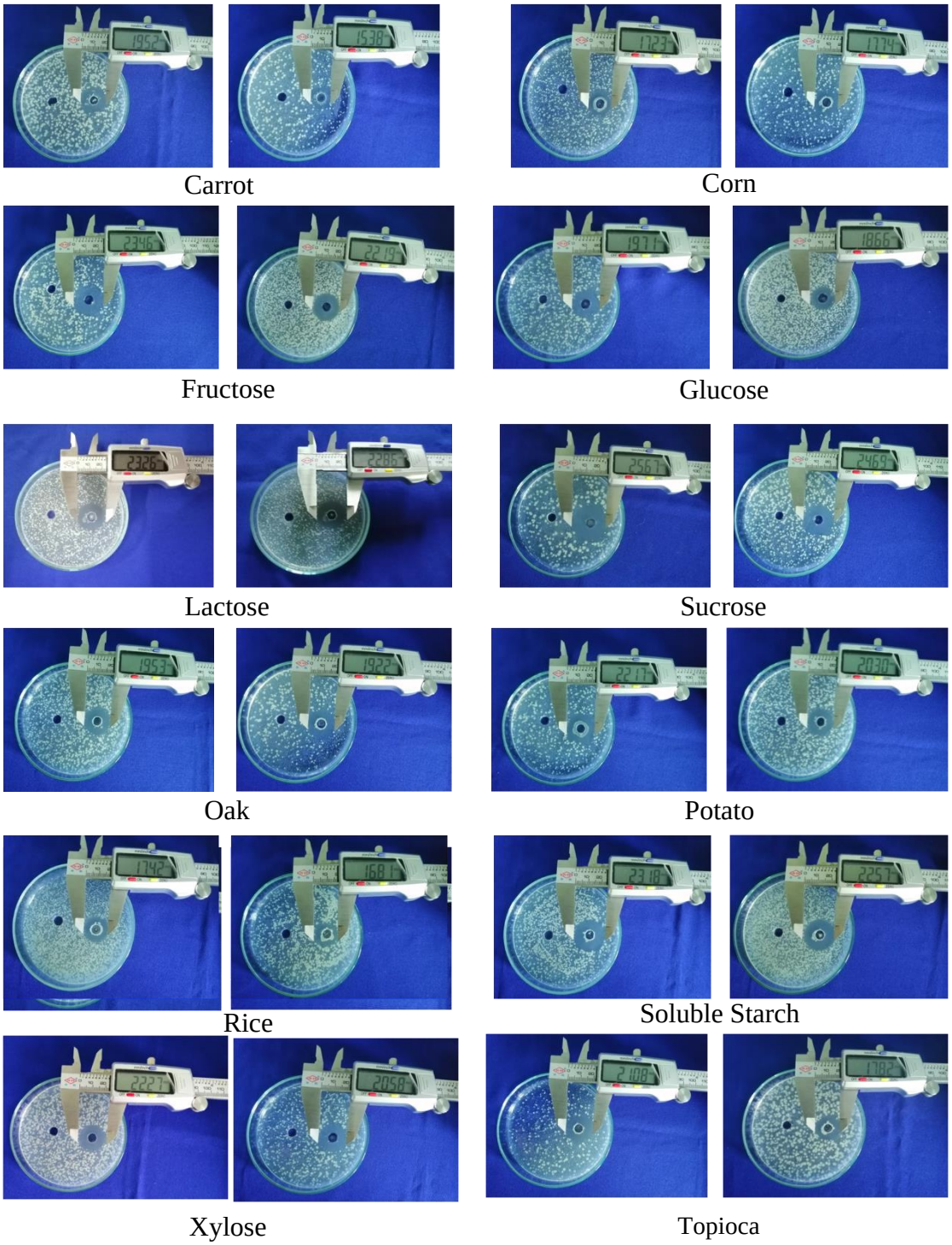


Fig. 3(a). Effect of Nitrogen Sources on selected strain against *Escherichia coli* and *Candida albicans*

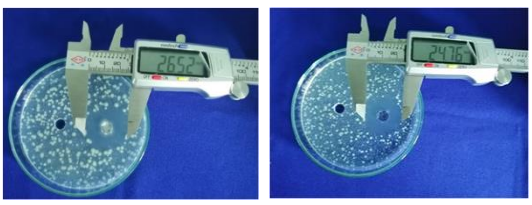


Fig.3(b). Effect of Nitrogen Sources on selected strain against *Escherichia coli* and *Candida albicans*

Effect of nitrogen sources on antimicrobial metabolite production of selected strain

Similarly, in the nitrogen source, the addition of yeast extract resulted maximum activity and inhibition zone reached (25.88 mm and 25.91 mm), followed by potassium nitrate (23.19 mm and 24.45 mm), ammonium nitrate (22.33 mm and 19.33mm), beef extract (21.85 mm and 18.86 mm) and gelatin (20.72 mm and 20.30 mm) against *Escherichia coli* and *Candida albicans.* These results have shown in Table 5 and Figure 4.

Table 5. The Effect of Nitrogen Source on the Fermentation of selected strain against *Escherichia coli* and *Candida albicans*

Sr. No	Nitrogen Source	Two test organisms and Inhibition Zone (mm)	
		Escherichia coli	***Candida albicans***
1	NH_4Cl	20.09	13.89
2	$NH_4\ NO_3$	22.33	19.33
3	$(NH_4)_2\ SO_4$	18.74	18.56
4	Caesin	18.44	20.87
5	Galetin	20.73	20.30
6	Malt Extract	20.25	18.09
7	Beef Extract	21.85	18.86
8	Milk	12.59	19.11
9	Peanut	16.82	16.80
10	Peptone	19.10	18.67
11	KNO_3	23.19	24.45
12	$NaNO_3$	19.83	17.54
13	Urea	19.33	18.59
14	Yeast Extract	**25.88**	**25.91**

12 -14 mm = weak activity, 15 - 19 mm = high activity,

>20 mm = very high activity (agar well size = 8 mm)

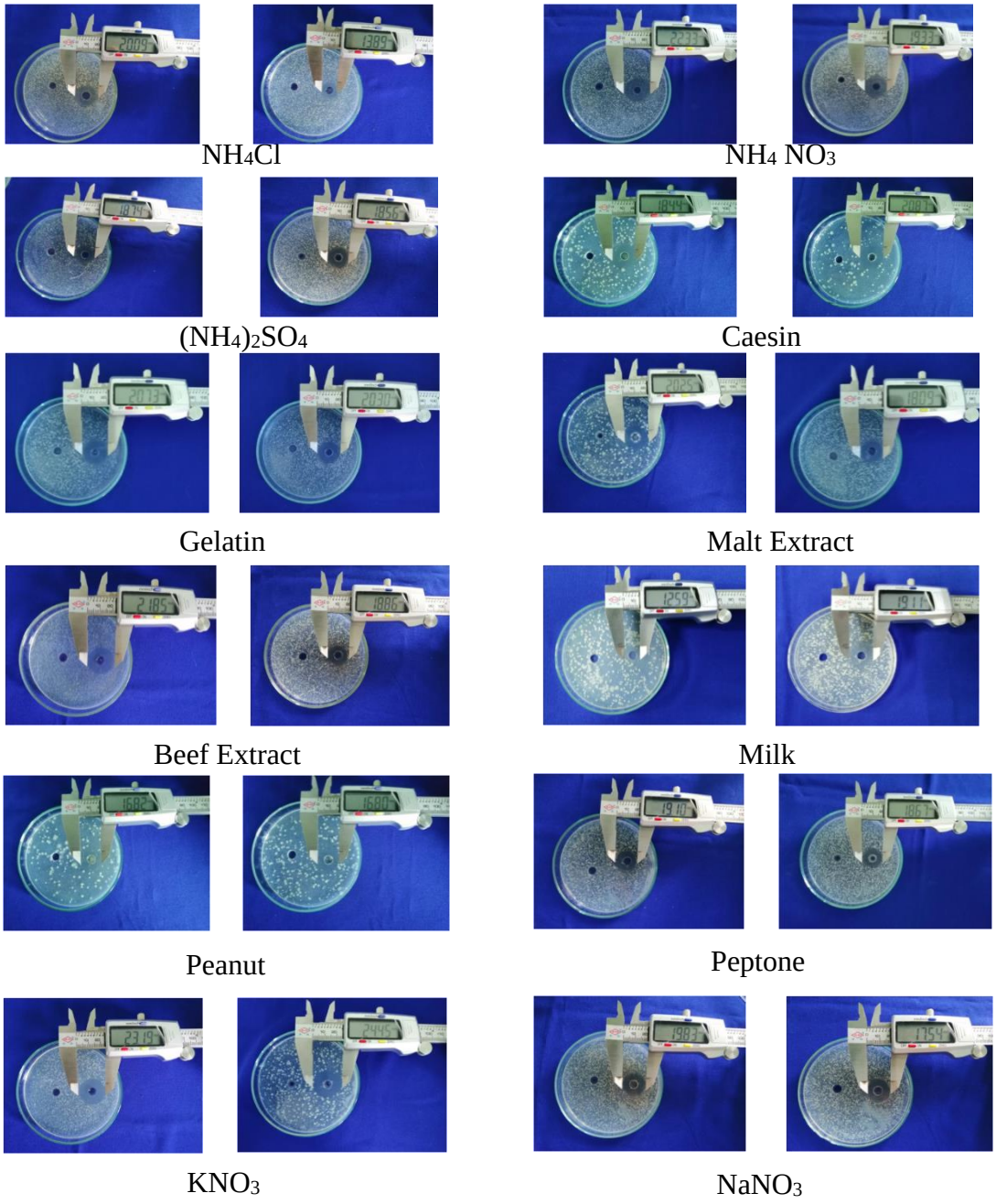


Fig. 4(a). Effect of Nitrogen Sources on selected strain against *Escherichia coli* and *Candida albicans*

Fig. 4(b). Effect of Nitrogen Sources on selected strain against *Escherichia coli* and *Candida albicans*

Effect of various fermentation media on antimicrobial metabolite production of selected fungus

In this research, optimization of fermentation medium of selected fungus were studied by various fermentation medium. In the fermentation medium (FM), TM-14 gave the highest antimicrobial activity at FM (6) (22.11 mm and 19.01 mm) followed by FM (12) (20.97 mm and 16.79 mm) and FM (10) (20.30 mm and 16.32 mm) on *E. coli*and *C. albicans* respectively. These results have shown in Table 6 and Figure 5.

Table 6. Selection of fermentation medium based on the results of antimicrobial activity

FM No.	Fermentation Media	Two test organisms and Inhibition Zone (mm)	
		E.coli	***C. albicans***
1	**Dextrose+Potassium Nitrate**	**20.17**	**16.00**
2	Fructose+Peptone	22.35	14.06
3	Glucose+Malt Extract	14.53	14.16
4	Glucose+Yeast Extract	15.06	16.44
5	Lactose+Yeast Extract	19.50	14.83
6	Maltose+Yeast Extract	22.11	16.64
7	Maltose+Casein	18.83	15.92
8	Maltose+Galetin	19.69	13.73
9	Maltose+Peptone	20.96	13.88
10	Maltose+Ammonium Nitrate	20.30	16.32
11	Potatose+Peptone	14.64	13.46
12	Soluble Starch+Beef Extract	20.97	16.79
13	Sucrose+Peptone	19.11	15.81
14	Sucrose+Yeast Extract	17.08	15.66
15	Xylose+Yeast Extract	19.43	19.01

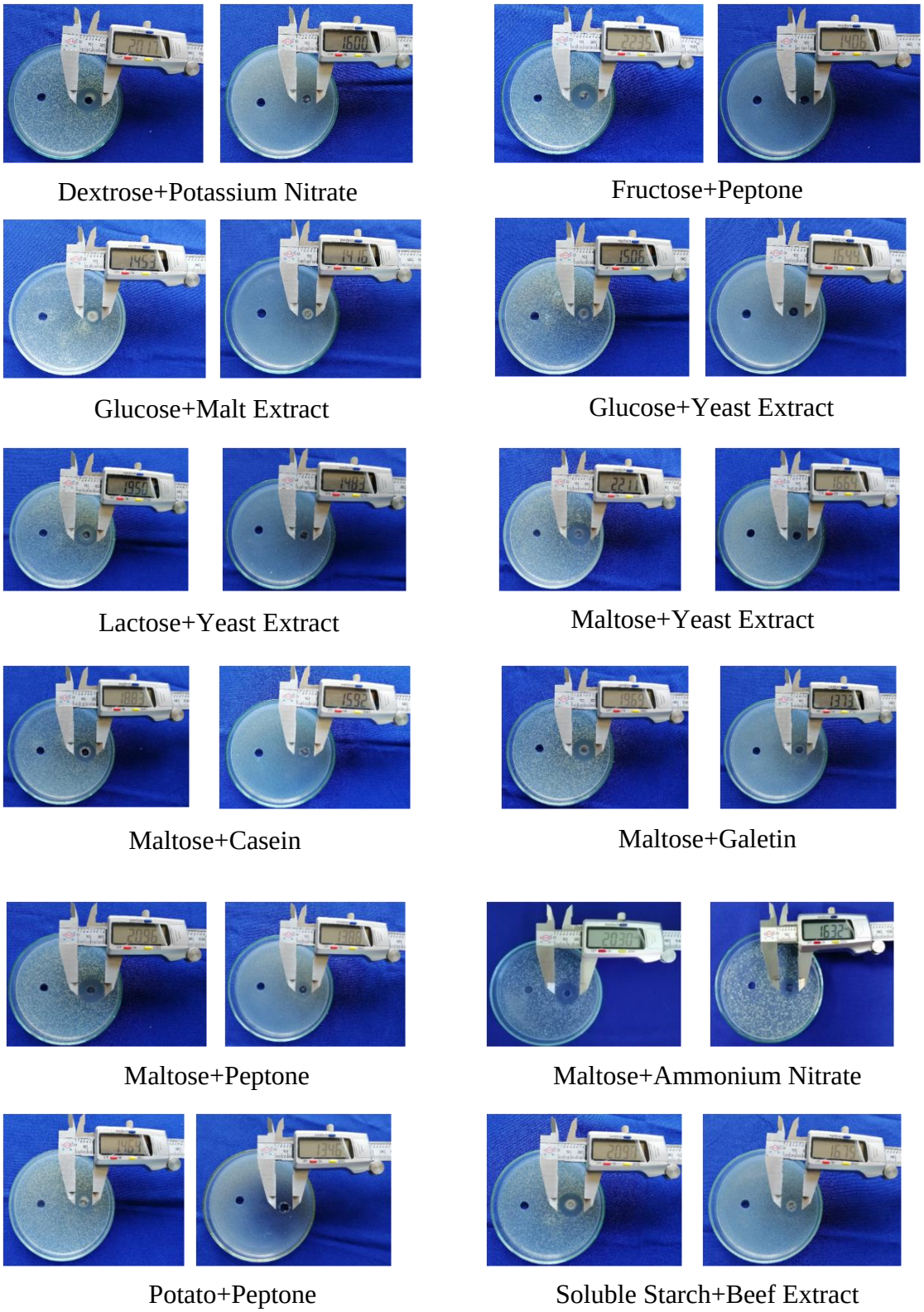
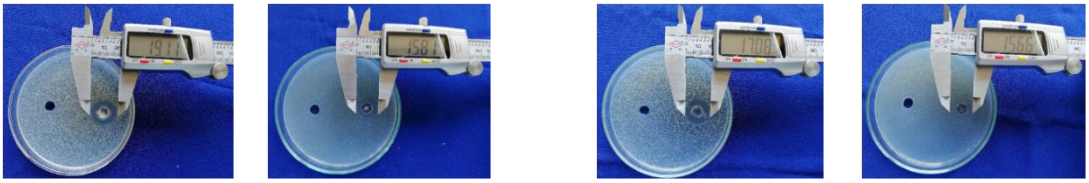
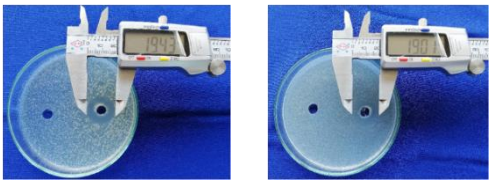


Fig. 5.(a) The antimicrobial activity of selected fermentation medium on *E. coli* and *C. albicans*



Sucrose+Peptone Sucrose+Yeast Extract



Xylose+Yeast Extract

Fig. 5.(b) The antimicrobial activity of selected fermentation medium on *E. coli* and *C. albicans*

Discussion And Conclusion

Qualitative and Quantitative aspects of antibiotic production by the microorganisms are dependent on the selective environmental pressure prevailing at its source of isolation and manipulation of growth and nutritional conditions during fermentation exerts substantial influence on the level of metabolite production (Yarbrough, 1993).

Optimal fermentation conditions such as proper age and size of inoculum are very important for the production of metabolites (Omura 1985).

The biosynthesis of antibiotics is regulated by the type and concentration of different medium components such as carbon, nitrogen, phosphate, metal ions and other medium ingredients (Martin and McDaniel, 1977).

The present study was focused on the fermentation conditions of selected fungal strain on *Escherichia coli and Candida albicans.* In the investigation to optimize the fermentation, it was found that 120 hrs of age of inoculum and 25 % of size of inoculum were suitable for fermentation of selected fungus. Studies carried out by some investigators revealed that maximal production of antibiotic substances occurred after 96 hrs (Reddy & Ranganathan, 1985; Sujatha *et al.,* 2005; Mustafa, 2009).

The highest biomass and antibiotic activity was observed at an incubation time of 72 hrs by some other investigators (Srinivasulu *et al.,* 2002).

In commercial industrial fermentation processes, it is well known that the age and density of the inoculum used directly influences on the duration of the lag phase, specific growth rate, biomass yield, sporation and quality of the final product (Stanburry *et al*, 1995).

The inoculum age and density markedly influence the productivity and economics of bioprocesses (Ramkrishna and Swaminathan, 2004).

The effect of carbon source on growth and antibiotic production is dependent upon several factors such as carbon concentration (Chen *et al.,* 2008).

In the effect of carbon and nitrogen sources, the highest antimicrobial activity of strain was found on maltose and yeast extract. Different carbon sources, like maltose (Fuji *et al.,* 1997), fructose (James *et al.,* 1988), xylose (Lowe *et al.,* 1997), dextrose (Rizk *et al., 2007*), lactose (Petersen *et al.,* 1994) and sucrose (Charkrabarti

et al., 1992) have been reported to be suitable for production of secondary metabolites in different microorganisms.

Sujatha (2005) reported that potassium nitrate indicated high activity and Yee Yee Thu (2006) reported that glucose and yeast extract media indicated high activity against *C. albicans*, *E. coli* and *S. aureus*. Fifteen kinds of fermentation media (FM) were utilized and the highest antimicrobial activity was obtained in FM-6. It was concluded that the current fermentation conditions that regulate antimicrobial substances metabolism and allow to choose fermentation process that minimize, moderate or maximize antimicrobial substances productivity. The future studies are expected extraction and identification of soil fungi.

Acknowledgements

First of all, I would like to express my thanks to U Aung Myat Soe, Principal, Yankin Education Degree College for her encouragement. I'm very grateful thanks to my supervisors, Dr. Zar Zar Yin, Professor, Department of Botany, Yenangyaung University and Dr. Ei Ei Khaing, Professor, Department of Chemistry, University of Pathein for their valuable instruction, constructive suggestion, insightful supervision for the successful completion of this research paper.

Reference

Ando K.M, Suto and Inada S. 2004. **Sampling and isolation methods of fungi**, workshop at university of Pathein.

Blakeslee, A. 1915. **Lindner's roll tube method of separation cultures.** Phyto-pathology 5: 68-69.

Charkrabarti S, Chandra AL. 1982. **A new streptomycete and a new polyene antibiotic acmycin.** *Folia icrobiol.* 27, 169-172.

Collin, C.H. 1965, **Microbiological Methods.** Butfer worth and Co., Publishers Ltd., Landon.

James P.D.A, Edwards C. 1998. **The effect of culture conditions on growth and Secondary metabolism in *Streptomyces thermoviolaceous* grown on chemostat** *FEMS Microbial. Letters.* 52, 1-6.

Lowe, S.E., Gustavson, D.R., Meyers. D.S., Vietch, J.A., Forenza, S. &Kam, K.S.(1997). **The effect of carbon source,temoerature and aeration on the production of Ascosteroside, a novel antifungal agent, by Ascotricha.** Journal of Antibiotics, 50, 412-417.

Luzhetskyy, A., Pelzer, S, and Bechthold, A. 2007. **The future of natural as a Source of new antibiotics.** Current Opinion in Investigational Drugs. 8: 608-613.

Magnuson, J. K. And Lasure, L.L. 2004. **Organic acid production by filamentous fungi.** In advance in fungal Bio-technology for industry, Agriculture and Medicine 9

Martin, J.F. McDaniel, L.E.1977. **Production of polyene macrolide antibiotics.** Advances in Applied Microbiology, 211-52.

Mustafa, O. 2009. **Antifungal and antibacterial compounds from Streptomyces strains.** *African Journal of Biotechnology* 8(13), 3007-3017.

Omura, S. 1984. **Microbial growth kinetics and secondary metabolites**, J. Fermentation Technology, 46:134-140

Petersen F, Moerker T, Vanzanella F, Peter HH. 1994. **Production of cladospirone bisepoxide, a new fungal metabolite.** *J. Antibiot.* 47, 1098-1103.

Ramkrishna, S., &Swaminathan, T. 2004. **Response surface modeling and optimization to elucidate and analyze the effects of inoculums age and size on surfaction production.** Biochemical Engineering Journal, 21 (2), 141-148.

Reddy, N.S., & Ranganathan, B. (1985). **Effect of time, temperature and pH on the growth and production of antimicrobial substance by *Streptococcus lactis* sp.** Diacetylactis SI- 67-C Milchwissenshaft, 40, 346 – 348.

Rizk MT, Metwally H. 2007. **Factors affecting growth and antifungal activity od some Streptomyces species against *Candida albicans.*** *J. Food Agric. Environ.* 5, 446-449.

Srinivasulu, B., Prakasham, R. S., Annapurna, J., Srinivas, S., Ellaiah, P., Ramakrishna, S.V. 2002. **Neomycin production with free and immobilized cells of *Streptomyce marinensis* in an airlift reactor,** Process Biochem. 38, 593-598.

Stanburry PF, Whitatker J, Hall SJ. **Principles of Fermentation Technology.** Oxford: Pergamon, 1995.

Stanburry PF, Whitatker J,Hall SJ. 1997. **Media for industrial fermentation In Principles of Fermentation Technology.** Oxford, UK, pp 93 – 116

Sujatha, P., Bapi Raju, K.V.V.S.N.,& Ramana, T.2005. **Studies on a new marine streptomycete BT-408 producing polyketide antibiotic SBR-22 effective against methicillin resistant *Staphylococcus aureus*.** Micro- biological Research, 160, 119-126.

Yarbrough, G.G. 1993. **Screening microbial metabolites for new drugs, theoretical and practical issues.** *Journal of antibiotics*, 46, 535-544.

Yee Yee Thu. 2006. **Novel Antimicrobial Metabolites Produced by *Trichoderma* sp., *Streptomyces* sp. and *Chaetomium* sp. Isolated from *Mimusops elengi* L., Soil and *Tamarix ananriensis* Willd**., Ph.D Thesis; Department of Botany, University of Yangon.