FERMENTATION CONDITIONS FOR THE ANTIBACTERIAL METABOLITES FROM ENDOPHYTIC FUNGUS YF-16

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Abstract

In the course of the isolation of fungi, twenty-nine fungi were isolated from five plant samples collected at Kangyidaung Township. The isolation of fungi was undertaken by surface sterilization method using LCA medium. Among them showed higher activity than other fungi. Therefore, these fungi were selected for further investigation. In the screening program for antimicrobial activity against *Staphylococcus aureus.* In the screening of antibacterial activity possessing microorganisms, isolated fungus YF-16 was highest activity reached at 3 days fermentation with 84hrs age and 10% size of inoculum. The selected fungus YF-16 was highest activity showed at FM2 medium.

Keywords Age and Size, Antibacterial activity, Fermentation

Introduction

Fermentation is a process which is usually influenced by different environmental parameters (Emily *et al*, 2009, Singh *et al*, 2017). Age and size of inoculum, temperature and pH are usually considered as most important parameters for microbial growth and production of value added products. Inoculum age and size also plays an important role in metabolite production (Xiaobo *et al*, 2006). The growth of microorganisms in the starter culture with respect to the time duration is crucial by using the inoculum (Emily *et al*, 2009).

Medium optimization is still one of the most critically investigated phenomenon that is carried out before any large scale metabolite production, and possess many challenges too. For designing a production medium, the most suitable fermentation conditions (e.g., pH, temperature, agitation speed, etc.) must be identified and optimized accordingly. Further, by optimizing the above said parameters, maximum product concentration could be achieved (Gupte and Kulkarni, 2003; Franco-Lara *et al*., 2006; Xiaobo *et al*, 2006; Wang *et al.*, 2011).

Therefore, the effects of carbon and nitrogen for the fermentation was investigated to produce antibacterial metabolite against *S. aureus*. Microbial fermentation has several advantages over using parts of the plants for the production of drugs and bioactive substances as this can easily be carried out in tank fermenters, providing unlimited supply of drugs and negating the requirement of plant parts. Moreover, different stronger derivatives of the drugs can be obtained by altering the culture conditions. Plants and microbes produce secondary metabolites with various biological activities that can treat various diseases (Radji, 2005).

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Materials and Methods

Investigation on Factors Affecting for the fermentation

1. Age and Size of Inoculum for the fermentation

2. Medium optimization for the fermentation

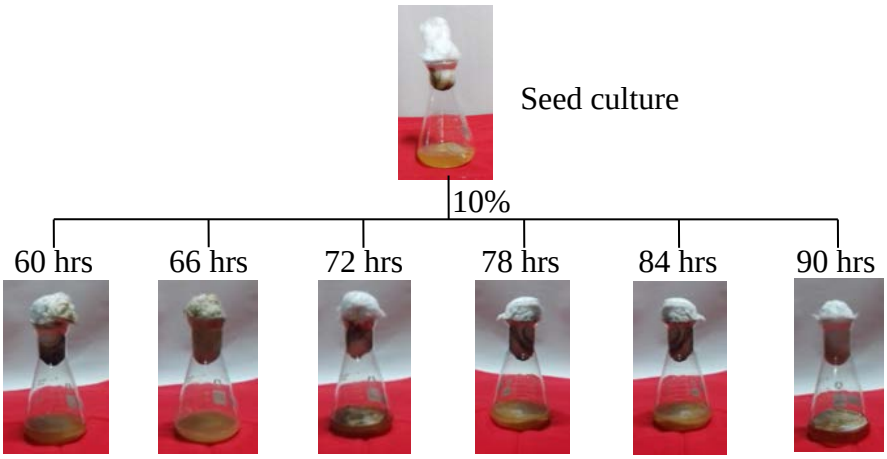
Study on Age and Size of Inoculum for the Fermentation

Fermentation is a process which is usually influenced by different environmental parameters (Emily *et al*, 2009). Inoculum age and size also plays an important role in metabolite production. Therefore, on the basis of the results of microbial growth kinetics, age of inoculum (60, 66, 72, 78, 84 & 90 hrs) with 10% seed culture was conducted for the fermentation to produce the metabolite. In the investigation of sizes of inoculum, 2.5 %, 5.0%, 7.5 %, 10.0%, 12.5 % and 15.0% were utilized with 84 hrs for YF-16 ages of culture.

Seed Medium (NITE, 2005)		**Fermentation Medium (NITE, 2005)**	
Glucose	1.0 g	Glucose	1.0 g
Glycerol	1.0 g	Glycerol	1.2 g
Yeast extract	0.8 g	Peptone	0.6 g
Polypeptone	0.7 g	$NaNO_3$	0.8 g
K_2HPO_4	0.001g	$MgSO_4.7H_2O$	0.001 g
$MgSO_4.7H_2O$	0.001g	K_2HPO_4	0.001 g
DW	100 mL	DW	100 mL
pH	6.5	pH	6.5

Assay Medium

Glucose	1.0 g
Yeast extract	0.2 g
DW	100 mL



Fermentation flasks with seed medium

Figure 1 Procedure for the study on the effects of YF-16 ages of inoculum

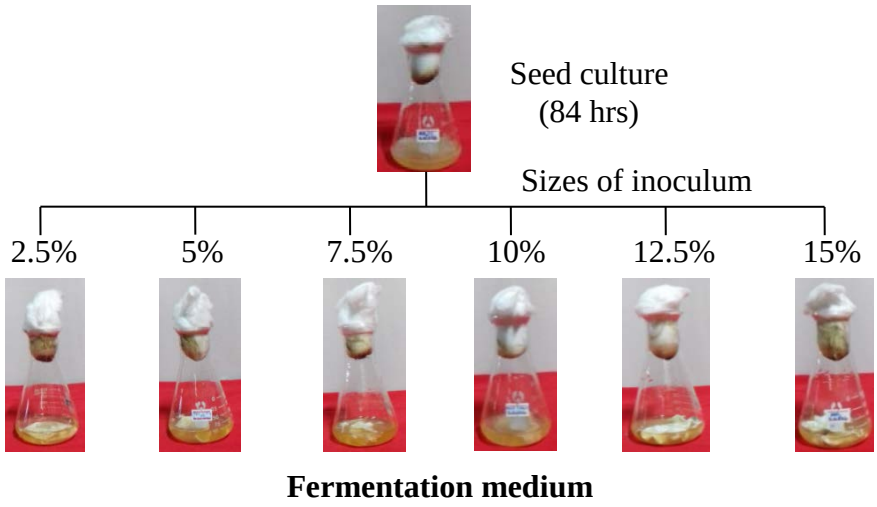


Figure 2 Procedure for the study on the effects of size of seed culture

Medium Optimization for fermentation and production of Antibacterial Metabolite

Fermentation was undertaken with suitable conditions of 84 hrs age of inoculum and 10 % sizes of inoculum with five different fermentation medium.

NITE (2005)

Seed medium	
Glucose	1.0 g
Yeast extract	0.5 g
Polypeptone	0.3 g
K_2HPO_4	0.001 g
$MgSO_4.7H_2O$	0.001 g
DW	100 mL
pH	6.5

Assay medium	
Glucose	1.0 g
Yeast extract	0.2 g
DW	100 mL

Fermentation Media (NITE 2005)

FM-1		**FM-2**		**FM-3**	
Glucose	1.0 g	Glucose	1.0 g	Glycerol	1.0 mL
Yeast extract	0.8 g	Sucrose	1.0 g	Yeast extract	0.8 g
Polypeptone	0.8 g	Yeast	1.5 g	Peptone	1.0 g
Glycerol	1.0 mL	K_2HPO_4	0.001 g	Tapioca powder	1.2 g
K_2HPO_4	0.001 g	DW	100 mL	K_2HPO_4	0.001 g
DW	100 mL	pH	6.5	DW	100 mL
pH	6.5			pH	6.5

FM-4

Glycerol	1.5 mL
Sucrose	1.8 g
Fish cake	1.8 g
K_2HPO_4	0.001 g
DW	100 mL
pH	6.5

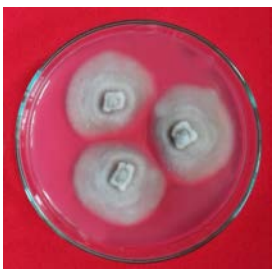
FM-5

Glucose	1.5 g
Molasses	1.5 g
KNO_3	0.5 g
Yeast extract	0.8 g
K_2HPO_4	0.001 g
DW	100 mL
pH	6.5

Results

Table 1 Fungi isolated from five medicinal plant samples by Surface sterilization Method

Sample	Surface Sterilization Method	
	Total Isolated Fungi	**Fungi No**
Clerodendrum indicum L. Kuntz	8	YF-02,02,03,04,05,06,07,08
Plumbago zeylanicaL.	7	YF-09,10,11,12,13,14,15
Sesbania grandiflora (L) Poret.	5	YF-16,17,18,19,20
HIppobroma longiflora (L) G Dong	4	YF-21,22,23,24
Dregea volubilis(L.f) Bth.ex.hook	5	YF-25,26,27,28,29
Total Isolated Fungi	29	



Front view

Reverse view

Front view - gray, Review - gray, Spore color - gray

YF-16 was isolated from leaf of *Sesbania grandiflora* (L.) Poiret.

Figure 3 Morphology of fungus YF-16 (7 days old culture on PGA medium)

Table 2 The effects of age of inoculum for the fermentation

Seed Culture (hrs)	Activity (mm)
60	25.73
66	27.10
72	29.18
78	30.06
84	33.51
90	29.76

These activities are three days fermentation from initial of fermentation.

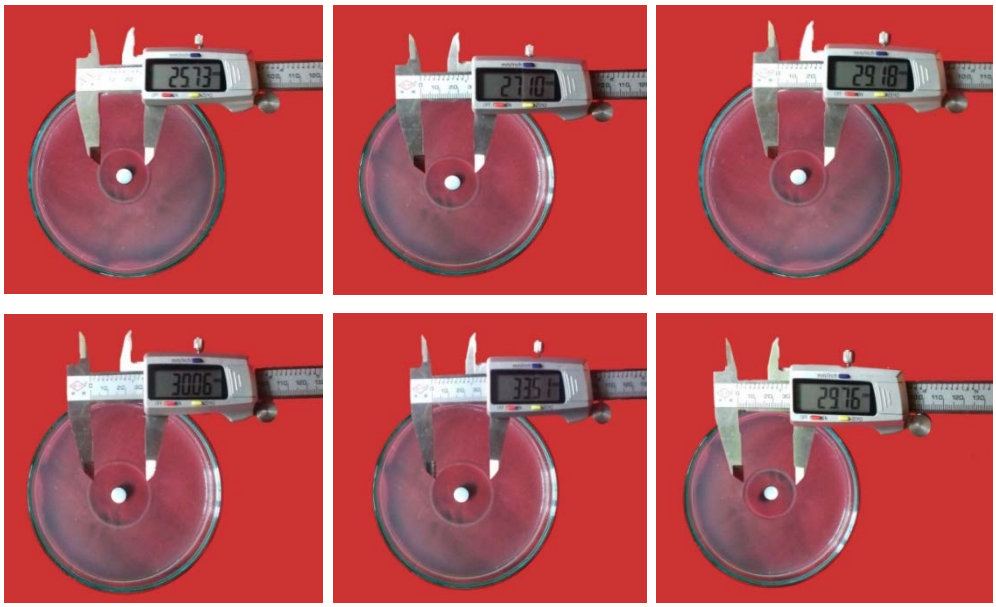


Figure 4 The Effects of Ages of Inoculums on Fermentation by YF-16 against *Staphylococcus aureus*

Table 3 The effects of size of inoculum for the fermentation

Seed Culture (%)	Activity (mm)
2.5	27.60
5.0	29.18
7.5	30.13
10.0	30.86
12.5	25.79
15.0	22.66

These activities are three days fermentation from initial of fermentation.

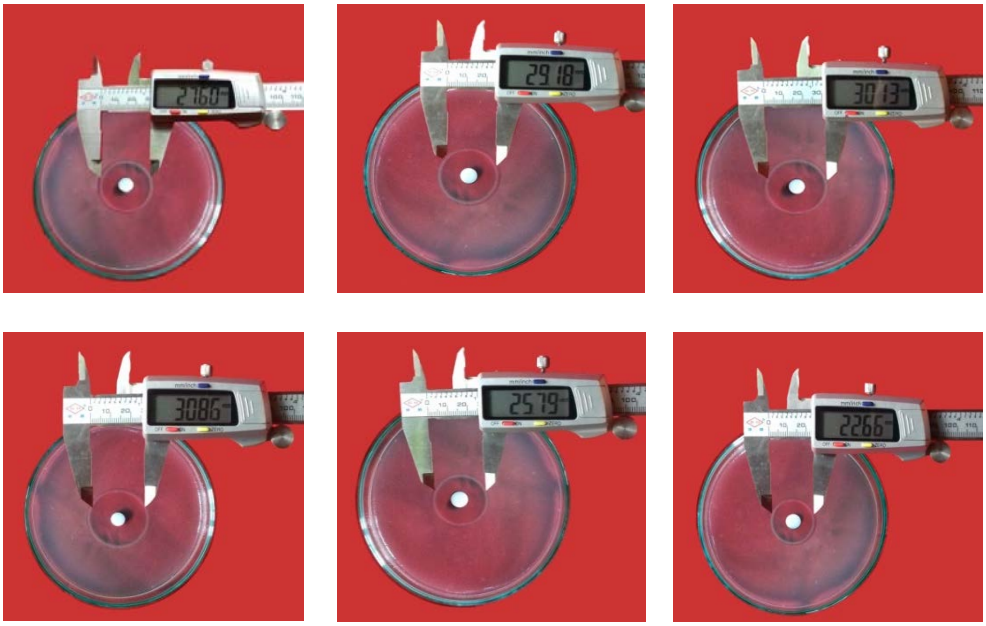


Figure 5 The Effects of Sizes of Inoculums on Fermentation by YF-16 against *Staphylococcus aureus*

FM-1 FM-2 FM-3 FM-4 FM-5

Figure 6 The media for the fermentation of antibacterial activity against *Staphylococcus aureus*

Table 4 The effect of media for the fermentation antibacterial activity against *Staphylococcus aureus*

Fermentation media	Antibacterial activity (clear zone, mm)
FM-1	31.11
FM-2	37.30
FM-3	26.53
FM-4	29.86
FM-5	30.68

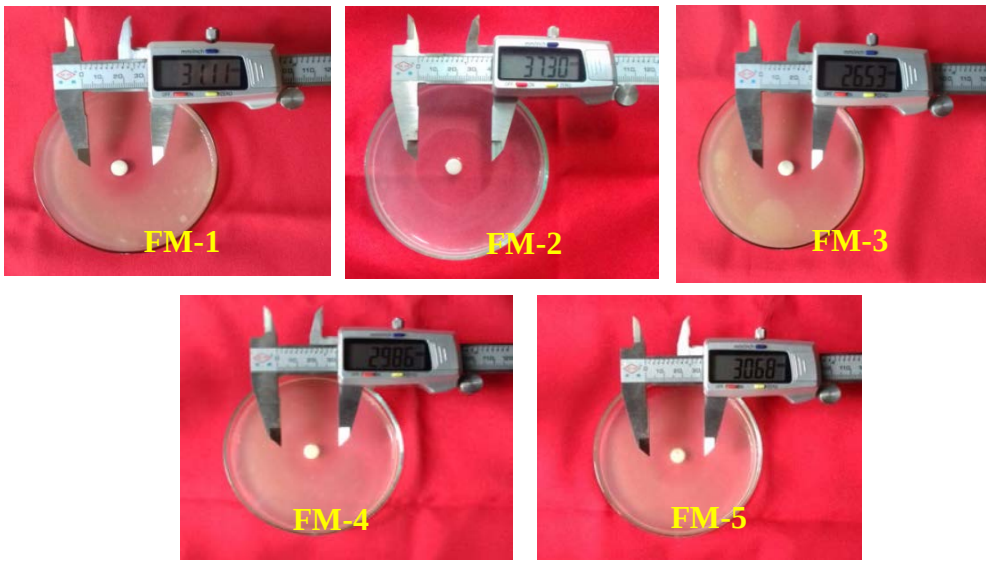


Figure 6 The effect of media for the fermentation of YF-16 Antibacterial activity against *Staphylococcus aureus*

Discussion and Conclusion

In the course of the isolation of fungi, twenty nine fungi were isolated from five plant samples collected at Kangyidaung Township. Eight fungi were isolated from *Clerodendrum indicum* L. Kuntz, seven fungi were isolated from *Plumbago zeylanica* L., five fungi were isolated from *Sesbania grandiflora* (L.) Poiret. Engl. and four fungi were isolated from *Hippobroma longiflora* (L.) G. Don., Five fungi were isolated from *Dregea volubilis* (L. f) Bth. ex.hook. Among them, fungus YF-16 exhibited the highest antibacterial activity against *S. aureus* because the test organism, Staphylococcus aureus is Methicilline (& other antibiotics) resistant bacterium that this fungus was selected for further investigations. In the study of time course of fermentation to produce the metabolite, the highest activity reached at 3 days fermentation as against *S. aureus* (33.51 mm) with 84hrs age and (30.86 mm) 10% size of inoculum. In this study FM2 medium is the highest activity for the production of metabolites. The proper cultivation (Ages) and transfer (Sizes) of inoculum is crucial for the production of both primary and secondary metabolites.

According to above mentioned results, time course of fermentation, extraction and purification of metabolite and bio-chemical properties will be undertaken.

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